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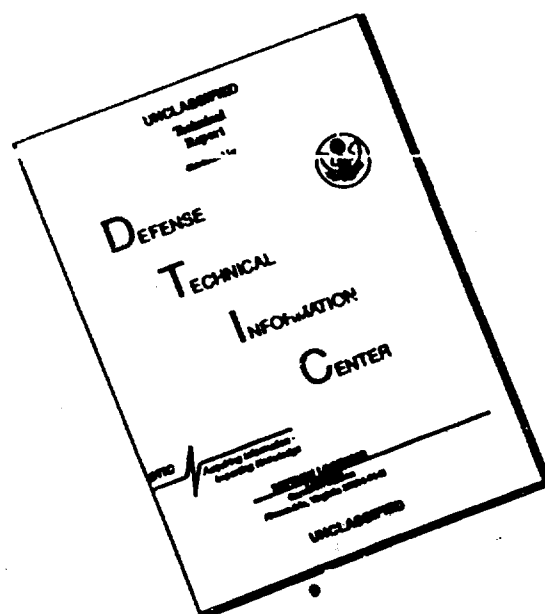
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THE 38TH ANNUAL MEETING
OF THE AMERICAN SOCIETY OF
TROPICAL MEDICINE AND HYGIENE

HILTON HAWAIIAN VILLAGE
HONOLULU, HAWAII
December 10 - December 14, 1989

PLEASE BRING THIS COPY TO THE MEETING

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* - The American Society of Tropical Medicine and Hygiene is certified by the Accreditation Council for Continuing Medical Education, Chicago, IL.

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PREPARATION OF THE PROGRAM BOOKLET

The General Program of the 38th Annual Meeting of the American Society of Tropical Medicine and Hygiene was prepared by American Institute of Biological Sciences, 730 11th Street, N.W., Washington, D.C. 20001-4584, Telephone 202/628-1500. Production by Louise Salmon (Meetings Manager), Adele V. Orndorff and Jean H. Cvetko.

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REGISTRATION INFORMATION

Place: Coral Lounge Office area, Mid-Pacific Convention Center, Hilton Hawaiian Village.

Times: Beginning Saturday, December 9, 1989, the registration area will be open from 12:00 noon to 5:00 PM. On Sunday, December 10, the registration area will be open from 8 AM to 8 PM. On Monday through Wednesday, December 11 through December 13, the registration area will be open from 8:00 AM to 5:00 PM. In addition, on Thursday, December 14, the registration desk will be open both for late registration and CME credits.

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TIME AND PLACE OF EXHIBITS

Place: Coral Ballroom I and II, Mid-Pacific Convention Center, Hilton Hawaiian Village.

Times:

Monday	11 December	10:00 AM - 5:00 PM
Tuesday	12 December	8:00 AM - 5:00 PM
Wednesday	13 December	8:00 AM - 5:00 PM
Thursday	14 December	8:00 AM - 12:00 Noon

* - As of October 19, 1989.

TOURS

Information and arrangements for tours and other sightseeing activities in the islands are available through the Hilton Hawaiian Village Hotel. Please contact the front desk or concierge. Arrangements are also available through Hilton Tours at their desk located in the meeting registration area, Coral Lounge, of the Mid-Pacific Convention Center. Additional information may be found in the Hospitality Suite located in the Nautilus I Room of the Mid-Pacific Convention Center.

AUDIOVISUAL FACILITIES

Slide projectors and carousels together with other audiovisual facilities will be available in the South Pacific Board Room, Mid-Pacific Convention Center, beginning at 3:00 PM on Sunday, December 10 and continuing (7:00 AM - 5:00 PM) until 12:00 noon on Thursday, December 14. These facilities are available for presenters to preview their slides. Investigators are requested to preview their slides and place them in carousels AT LEAST ONE DAY IN ADVANCE of their scientific presentation. The South Pacific Board Room is next to Sea Pearl I and across the hall from South Pacific I.

Videotape facilities will also be available on request in the South Pacific Boardroom. Please specify either BETA or VHS and provide at least 24 hours notice.

MICROSCOPE FACILITIES

A compound microscope will be available in Sea Pearl I, Mid-Pacific Convention Center beginning at 4:00 PM on Sunday, December 10 and continuing (8:00 AM - 5:00 PM) until 12:00 noon on Thursday, December 14.

ASTMH ARCHIVES

The ASTMH Archives will be on display in Sea Pearl I, Mid-Pacific Convention Center. The Archives Exhibit was created by Linda Brink, Wilbur Downs, Richard Krause and Charles Morrissey. It reviews the historical influences of various government agencies and philanthropic organizations on U.S. Tropical Disease programs and initiatives. Research for the exhibit was sponsored by the Rockefeller Archives and Fogarty International Center.

The Archives will be open for viewing from 8:00 AM to 5:00 PM beginning at 1:30 PM on Monday and concluding at 5:00 PM on Wednesday. The prototype "Global Epidemic Emergency" (GEE) interactive model was developed by Frank Toth and his colleagues from the Center for Interactive Media in Medicine at USUHS. Society members are invited to use the model to review the parameters of the GEE scenario (at the Archives in Sea Pearl I) and to update the system by adding factual information related to their own specialties. Dr. Toth will be available at the Archives on Monday, December 11 to discuss the potential of interactive media and to demonstrate several interactive medical training models developed at USUHS.

HOSPITALITY SUITE

A Hospitality Suite for the spouses, friends and family of members attending the meeting will be located in Nautilus I, Mid-Pacific Convention Center. It will be open from 2:00 to 5:00 PM on Sunday, December 10 and from 8:00 AM to 5:00 PM, December 11-14.

EMERGENCY CALLS AND MESSAGES

Emergency calls should be directed to (808) 949-4321, extensions 74240 or 75241.

A message board will be available across the registration desk, Coral Lounge, Mid-Pacific Convention Center.

EMPLOYMENT OPPORTUNITIES

Two bulletin boards will be available across from the registration desk in the Coral Lounge, Mid-Pacific Convention Center.

NOTICES

Badges must be worn to attend all functions.

Smoking will be permitted only where specifically authorized. This rule is in compliance with the Resolution on Smoking that was adopted at the ASTMH Annual Business Meeting on November 5, 1976. The cooperation and thoughtfulness of smokers is requested to minimize embarrassment and discomfort for all persons.

The time and/or location of all activities are subject to change. Change notices will be posted in the Registration Area.

PROGRAM NOTES

The Symposia are intended to provide updates on subjects which have changed significantly in the last several years. They are designed especially for members who are not working actively in those areas, and often focus on work that has been published previously.

In contrast, the scheduled scientific sessions and the Late-Breaking Advances in Molecular Biology Workshop focus on recent unpublished results. Investigators interested in presenting in the Late-Breaker Molecular Biology session should contact Dr. Michael R. Hollingdale (301-881-3300) or Dr. Steven R. Meshnick (212-690-6628) within 3 weeks of the meeting or by noon on Monday, December 11 and come prepared to make a 10 minute oral presentation.

Oral presentations (in the regular sessions) should be 10 minutes or less in length unless noted otherwise, with 5 minutes for questions and discussion. Poster presentations are also based on recent unpublished work, but provide more time for informal discussion. The posters may be set up beginning at 7:00 PM the night before, but should be assembled by 8:00 AM. Authors should be in attendance from 8:00 AM to 10:00 AM at each poster session. Posters should be taken down by 5:00 PM on both Tuesday and Wednesday.

Suggestions for Changes in the meeting should be directed to the members of the Scientific Program Committee.

LOCAL COMMITTEE BUSINESS OFFICE AND INFORMATION CENTER

The Registration Area will be used as a business office for the Local Committee after the initial registrations have been completed on Monday, December 11.

CONTINUING EDUCATION FOR PHYSICIANS

The American Society of Tropical Medicine and Hygiene is approved by the Accreditation Council on Continuing Medical Education to certify Category I CME credits for scientific portions of the Annual Meeting (up to 30 hours). Attendees who desire CME credit must pay a documentation fee of \$30 and must have paid their registration fee for the meeting. Each participant must return the CME Registration Form and the Attendance and Evaluation Form to the Office-Registration Area on the Coral Ballroom Level by the end of the meeting. Certificates based on the attendance recorded on the Attendance and Evaluation Form will be mailed to participants within one month of the meeting.

CLINICAL TROPICAL MEDICINE CME COURSE

SATURDAY, DECEMBER 9, 1989

7:00 AM - 4:00 PM

South Pacific I-II

7:00	REGISTRATION: Outside the meeting room
7:30	CONTINENTAL BREAKFAST.
8:00	INTRODUCTION/WELCOME. Michele Barry.
8:05	GENERAL ADVICE FOR TRAVELERS. Martin S. Wolfe.
8:30	IMMUNIZATION. Kenneth R. Dardick.
9:10	MALARIA PREVENTION. Stephen L. Hoffman.
9:40	QUESTION PERIOD.
10:00	COFFEE BREAK.
10:30	TRAVELERS' DIARRHEA: ETIOLOGY, PREVENTION AND SELF-MANAGEMENT. Martin S. Wolfe.
11:10	JET LAG AND MOTION SICKNESS. Kenneth R. Dardick.
11:40	QUESTION PERIOD.
12:00	LUNCH -- SOUTH PACIFIC ROOMS III AND IV.
1:30	PREGNANT AND INFANT TRAVELERS. Michele Barry.
2:00	ELDERLY AND CHRONICALLY ILL TRAVELERS. Victor Rovner.
2:30	AIDS AND THE TRAVELER. David R. Hill.
3:00	HEAT, SUN EXPOSURE AND INSECT REPELLENTS. Michele Barry.
3:30	QUESTION PERIOD.
6:00	RECEPTION. Honolulu III - Lanai, Tapa Tower.

CLINICAL TROPICAL MEDICINE CME COURSE*
SUNDAY, DECEMBER 10, 1989

7:30 AM - 4:00 PM

South Pacific I-II

7:30	CONTINENTAL BREAKFAST
8:00	HIGH ALTITUDE TRAVEL. Frank Bia.
8:30	MEDICAL KITS, TRAVEL INSURANCE AND SOURCES OF INFORMATION. Victor Kovner.
9:00	FEVER AND MALARIA IN RETURNING TRAVELERS. Stephen L. Hoffman.
9:40	QUESTION PERIOD.
10:00	COFFEE BREAK.
10:25	HAZARDOUS SEAFOOD. Elaine C. Jong.
10:50	CLINICAL SYNDROMES IN RETURNING TRAVLERS. David R. Hill.
11:15	EOSINOPHILIA. Jay S. Keystone.
11:40	QUESTION PERIOD.
12:00	LUNCH -- SOUTH PACIFIC ROOMS III AND IV.
1:30	DIARRHEA IN RETURNING TRAVELERS. Leonard C. Marcus.
2:00	TROPICAL DERMATOLOGY. Jay S. Keystone.
2:30	DIAGNOSTIC PEARLS ABOUT PARASITES. Leonard C. Marcus.
2:55	NEW TREATMENTS FOR OLD PARASITES. Elaine C. Jong, MD.
3:15	QUESTION PERIOD.
3:35	CASE PRESENTATIONS. Frank Bia.
6:00	ASTMH RECEPTION. Lagoon Green.

* - To register for the course, contact Josephine Onofrio (203 785-2476).
Registration is \$250.00 for Physicians; \$150.00 for House Staff, Fellows
and Nurses; and \$50.00 for Students (please provide evidence of student
status).

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM AND OF SPECIAL EVENTS

FRIDAY, DECEMBER 8

6:00 PM - 8:00 PM	Registration for CME Course	Offices - Registration Coral Ballroom Level
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SATURDAY, DECEMBER 9

7:00 AM - 4:00 PM	CME Course in Clinical Tropical Medicine	South Pacific I-II
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7:30 AM	Continental Breakfast for CME Course Participants	South Pacific I-II
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12:00 NN - 1:00 PM	Lunch for CME Course Participants	South Pacific III-IV
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12:00 NN - 5:00 PM	ASTMH Registration	Offices - Registration Coral Ballroom Level
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6:00 PM - Evening	Reception for CME Course Participants	Honolulu III - Lanai Tapa Tower
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SUNDAY, DECEMBER 10

7:00 AM - 4:00 PM	CME Course in Clinical Tropical Medicine	South Pacific I-II
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7:30 AM	Continental Breakfast for CME Course Participants	South Pacific I-II
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8:00 AM - 5:00 PM	ASTMH Annual Council Meeting	Ti Leaf
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8:00 AM - 8:00 PM	ASTMH Registration	Offices - Registration Coral Ballroom Level
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12:00 NN - 1:00 PM	Lunch for CME Course Participants	South Pacific III-IV
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12:00 NN - 4:00 PM	SIRACA Subcommittee of ACAV	Nautilus II
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12:00 NN - 6:00 PM	ACME Council Meeting	Nautilus III
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4:30 PM - 6:00 PM	ACAV Council Meeting	Nautilus II
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6:00 PM - 8:00 PM	ASTMH Opening Reception (Badges Required)	Lagoon Green
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SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM AND OF SPECIAL EVENTS (Continued)

MONDAY, DECEMBER 11

7:00 AM	Editorial Board Meeting and Continental Breakfast - AJTMH	Ti Leaf
8:00 AM - 5:00 PM	ASTMH Registration	Offices - Registration Coral Ballroom Level
8:00 AM - 12:00 NN	ASTMH Opening Plenary Session	Coral III-IV
10:00 AM - 5:00 PM	Exhibits	Coral I-II
10:00 AM - 10:30 AM	Coffee Break	Coral I-II
12:00 NN - 1:30 PM	Lunch Break	
12:00 NN - 1:30 PM	Press Conference and Congressional Luncheon	Ti Leaf Sea Pearl IV-VI
1:30 PM - 4:30 PM	Symposium: Nutrition and Infection.	Coral III
1:30 PM - 2:15 PM	Tropical Medicine Commemorative Fund Lecture K.N. Mendis	Coral IV
2:15 PM - 5:15 PM	Scientific Session A: Malaria - Blood Stages.	Coral IV
1:30 PM - 4:30 PM	Scientific Session B: Virology - Diagnosis and Epidemiology.	South Pacific I-II
1:30 PM - 4:30 PM	Scientific Session C: Filariasis - Immunoregulation.	South Pacific III-IV
1:30 PM - 6:00 PM	5th Annual Meeting of the American Committee on Medical Entomology (ACME).	Nautilus II-III
3:00 PM - 3:30 PM	Coffee Break	Coral I-II
5:00 PM	American Committee on Medical Malacology, Open Organizational Meeting.	Sea Pearl II-III
5:00 PM - 6:00 PM	Seminar on the Legislative Process: Advocacy Workshop	South Pacific III-IV
6:00 PM - 8:00 PM	ASTMH Archives. Reception sponsored by the Archives Committee and the Legislative Task Force	Coral III

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM AND OF SPECIAL EVENTS (Continued)

TUESDAY, DECEMBER 12

7:00 AM - 8:00 AM	ASTMH Past Presidents' Meeting and Breakfast	Ti Leaf
7:00 AM - 8:00 AM	Scientific Program Committee Meeting and Breakfast	Nautilus I
7:30 AM - 10:00 AM	Poster Session I with Complimentary Continental Breakfast	Coral Lounge
8:00 AM - 5:00 PM	ASTMH Registration	Offices - Registration Coral Ballroom Level
8:00 AM - 5:00 PM	Exhibits	Coral I-II
10:00 AM - 12:00 NN	Symposium: Cytoadherence and Cerebral Malaria.	Coral III
10:00 AM - 12:00 NN	Scientific Session D: Clinical Tropical Medicine.	Coral IV
10:00 AM - 12:00 NN	Scientific Session E: Arboviral Entomology.	South Pacific I-II
10:00 AM - 12:00 NN	Scientific Session F: Schistosomiasis - Immunopathogenesis and Mechanisms of Immunity.	South Pacific III-IV
10:00 AM - 12:00 NN	Scientific Session G: Kinetoplastidia - Immunology.	Nautilus II-III
12:00 NN - 1:30 PM	Lunch Break	

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM AND OF SPECIAL EVENTS (Continued)

TUESDAY, DECEMBER 12

1:30 PM - 3:00 PM	Scientific Session H: Viral Pathogenesis,	South Pacific I-II
1:30 PM - 3:00 PM	Scientific Session I: Malaria - Molecular Biology.	South Pacific III-IV
1:30 PM - 3:00 PM	Scientific Session J: Giardiasis and Toxoplasmosis.	Nautilus II-III
1:30 PM - 3:00 PM	Scientific Session K: Tropical Veterinary Medicine.	Sea Pearl II-III
1:30 PM - 3:00 PM	Scientific Session L: Entomology - Sandflies.	Sea Pearl IV-VI
3:00 PM - 3:30 PM	Coffee Break	Coral I-II
3:30 PM - 4:30 PM	Presidential Address L.H. Miller	Coral III-IV
4:30 PM - 6:00 PM	ASTMH Annual Business Meeting	Coral III-IV

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM AND OF SPECIAL EVENTS (Continued)

WEDNESDAY, DECEMBER 13

7:30 AM - 10:30 AM	Poster Session II with Complimentary Continental Breakfast	Coral Lounge
8:00 AM - 5:00 PM	ASTMH Registration	Offices - Registration Coral Ballroom Level
8:00 AM - 5:00 PM	Exhibits	Coral I-II
10:00 AM - 12:00 NN	Scientific Session M: Late Breakers in Molecular Biology Workshop.	Coral III
10:00 AM - 12:00 NN	Scientific Session N: Molecular Virology.	Coral IV
10:30 AM - 12:00 NN	Scientific Session O: Entomology - Lyme Disease.	South Pacific I-II
10:00 AM - 12:00 NN	Scientific Session P: Ameliasis.	South Pacific III-IV
9:45 AM - 12:15 PM	Scientific Session Q: Tapeworms.	Nautilus II-III
12:00 NN - 1:30 PM	Lunch Break	
1:30 PM - 5:00 PM	Scientific Session R: Filariasis - Onchocerciasis and Ivermectin.	Coral III
1:30 PM - 5:15 PM	Scientific Session S: Malaria - Pre-Erythrocytic Stages.	Coral IV
1:30 PM - 5:30 PM	Clinical Tropical Medicine Group Meeting.	South Pacific I-II
1:30 PM - 5:00 PM	30th Annual Open Meeting of the American Committee on Arthropod-Borne Viruses (ACAV).	South Pacific III-IV
1:30 PM - 4:30 PM	Scientific Session T: Epidemiology.	Nautilus II-III
3:00 PM - 3:30 PM	Coffee Break	Coral I-II
6:00 PM - 7:00 PM	ASTMH Reception and No-Host Cocktail Party	Coral Lounge
7:00 PM - 10:00 PM	ASTMH Annual Banquet	Coral III-IV

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM AND OF SPECIAL EVENTS (Continued)

THURSDAY, DECEMBER 14

7:00 AM	ASTMH Council Meeting	Ti Leaf
8:00 AM - 5:00 PM	CME Certification	Offices - Registration Coral Ballroom Level
8:00 AM - 10:30 AM	Symposium: International Travel Medicine.	Coral III-IV
8:00 AM - 10:30 AM	Scientific Session U: Filariasis - Molecular Biology.	South Pacific I-II
8:00 AM - 10:30 AM	Scientific Session V: Virus Vaccines.	South Pacific III-IV
8:00 AM - 10:30 AM	Scientific Session W: Kinetoplastidia - Biochemistry, Molecular Biology and Chemotherapy.	Nautilus II-III
8:30 AM - 10:30 AM	Scientific Session X: Schistosomiasis - Antigens and Immunogens	Sea Pearl III-VI
8:00 AM - 12:00 NN	Exhibits	Coral I-II
10:30 AM - 11:00 AM	Coffee Break	Coral I-II
11:00 AM - 12:00 NN	Fred Soper Lecture W.H. Foegel	Coral III-IV
12:00 NN - 1:30 PM	Lunch Break	
1:30 PM - 5:00 PM	Symposium: Hansen's Disease.	Coral III
1:30 PM - 5:30 PM	Symposium: Malaria in Pregnancy.	Coral IV
1:30 PM - 2:45 PM	Scientific Session Y: Retroviral Infections.	South Pacific I-II
3:00 PM - 4:40 PM	Symposium: Opportunistic Infections in AIDS.	South Pacific I-II
1:30 PM - 5:00 PM	Scientific Session Z: Malaria Chemotherapy.	South Pacific III-IV
1:30 PM - 5:00 PM	American Society of Tropical Veterinary Medicine.	Nautilus II-III
3:00 PM - 3:30 PM	Coffee Break	Coral I-II

DETAILED SCIENTIFIC PROGRAM

Throughout the Scientific Program, an asterisk (*) denotes
the person presenting paper.

MONDAY MORNING, DECEMBER 11

ASTMH OPENING PLENARY SESSION:
A PROUD TRADITION, GRAVE NEW CHALLENGES*

The Plenary Session was organized by Linda Brink and Don Hopkins. It represents a continuation of the Society's response to America's declining capacity to address tropical disease problems.

Although the 1989 Plenary Session focuses on a hypothetical scenario, the potential disease problems are all too real. In sub-Saharan Africa, 200 million people are chronically infected with malaria. Malaria is responsible for the deaths of more than one million infants and young children each year. The mosquito vectors for malaria occur in many parts of the developed world. There is a resurgence of dengue and two million cases occur world-wide each year. Dengue hemorrhagic fever, a form of dengue lethal to children, claims thousands of lives in Southeast Asia. The mosquitoes which transmit dengue are present, once again, in the US. These are two examples of the many Third World diseases which could spread into susceptible populations in the US and elsewhere. As Dr. Robert Shope of Yale University School of Medicine, stated recently, "It is not a question of whether there will be a major disease outbreak, but when". The ASTMH 1989 Plenary Session illustrates the fine line between reality and a hypothetical situation.

SCENE: A meeting in the Surgeon General's Office
An Emergency Interagency Working Group (EIWG) has been convened in response to the "Global Epidemic Emergency", a situation which will severely strain US capabilities in tropical infectious diseases. The EIWG meeting is divided into two parts: 1) definition of the potential problems and 2) assessment of the ability of the US and international organizations to respond.

BACKGROUND:
The "Global Epidemic Emergency" takes place in late 1991, in a sub-Saharan African country (Changa), which has experienced a devastating civil war, and in its neighbors (Basangani and Lubawe). Peace-keeping forces, relief workers from many western countries (including the United States), and refugees fleeing Changa are reporting hepatitis, cholera, and unusual febrile illnesses. (The "unusual febrile illnesses" may represent cases of African hemorrhagic disease.) Military personnel returning to Fort Bragg are experiencing a "flu syndrome" -- fifteen have been hospitalized, and an additional forty soldiers have been placed on quarters. Other diseases known to be endemic in Changa include schistosomiasis, yellow fever, trypanosomiasis, AIDS, and typhus.

YOUR ROLE:
As an ASTMH member, you have been identified as a tropical disease expert and are requested to advise the EIWG. Please be prepared to provide an assessment of the clinical expertise, diagnostic, treatment, and prevention capabilities within your specialty. Refer to your BRIEFING PAPERS for further instructions.

* - The ASTMH Opening Plenary Session is supported by the US Army Medical Research and Development Command, the Rockefeller Foundation, the Rockefeller Archives and the Fogarty International Center.

MONDAY MORNING, DECEMBER 11

ASTMH OPENING PLENARY SESSION (Continued)

8:00 AM - 12:00 NN

Coral III - IV

Time Abstract

8:00 1 WELCOME AND DESCRIPTION OF THE PLENARY SESSION: L.H. Miller, President, ASTMH. National Institutes of Health, Bethesda, MD.

8:15 2 DESCRIPTION OF THE "GLOBAL HEALTH" SCENARIO AND THE OBJECTIVES OF THE EIWG. L.J. Legters, EIWG Chairperson. Uniformed Services University of the Health Sciences, Bethesda, MD.

8:30 DEFINITION OF THE PROBLEM

3 IN-COUNTRY SITUATION AND MILITARY OVERVIEW. E.T. Takafuji, Representing the Office of the Army Surgeon General. Office of the Army Surgeon General, Falls Church, VA.

4 GLOBAL IMPACT. M.S. Wolfe, Representing the Department of State. Department of State, Washington, DC.

5 U.S. PUBLIC HEALTH CONCERNS. D.R. Hopkins, Representing the National Institute for Allergy and Infectious Diseases and the Centers for Disease Control. Global 2000, Carter Center, Atlanta, GA.

9:30 DISCUSSION

10:00 COFFEE BREAK

10:30 POTENTIAL RESPONSES

6 THE GLOBAL HEALTH SITUATION. A.O. Lucas, International Health Expert, Representing the World Health Organization. Carnegie Corporation, New York, NY.

7 MEDICAL CAPABILITIES OF THE U.S. MILITARY. P.K. Russell, Representing the U.S. Military. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

8 THE U.S. CAPACITY TO ADDRESS TROPICAL DISEASE PROBLEMS. K.M. Johnson, Physician-Scientist, Representing the U.S. Civilian Sector. National Biological Systems, Rockville, MD.

9 THE CONGRESSIONAL RESPONSE. The Honorable Daniel K. Inouye, U.S. Senator from Hawaii (Invited).

11:30 DISCUSSION

MONDAY NOON, DECEMBER 11

12:00 Noon - 1:30 PM

Ti Leaf

PRESS CONFERENCE. ASTMH Spokespersons, Representatives of Congress and the Press.

Sea Pearl IV - VI

BUFFET LUNCH (following the Press Conference). L.H. Miller, J.K. Frenkel, S.R. Sagebiel, ASTMH Council, Plenary Session Speakers, ASTMH Members from Hawaii, Legislative Representatives and Members of the Press.

MONDAY AFTERNOON, DECEMBER 11

SYMPOSIUM: NUTRITION AND INFECTION*

1:30 PM - 4:30 PM

Coral III

Chairpersons: R.L. Guerrant and I. Kakoma

Time Abstract

1:30	10	MECHANISMS AND MEDIATORS OF MALNUTRITION AND TROPICAL INFECTIOUS DISEASES. A. Cerami. Rockefeller University, New York, NY.
2:00	11	VISCERAL LEISHMANIASIS: A MODEL FOR INFECTION-INDUCED CACHEXIA. R.D. Pearson. University of Virginia, Charlottesville, VA.
2:30	12	DIARRHEA AS A CAUSE OF MALNUTRITION. L. Mata. Institute for Health Research, University of Costa Rica, COSTA RICA.
3:00		COFFEE BREAK
3:30	13	MALNUTRITION AS A CAUSE OF DIARRHEA. R.L. Guerrant. University of Virginia School of Medicine, Charlottesville, VA.
4:00	14	A PRACTICAL STRATEGY FOR THE DEVELOPING WORLD. A.O. Lucas. Carnegie Corporation, New York, NY.

* - This Symposium on Nutrition and Infection is supported by the Agency for International Development.

SCIENTIFIC SESSION A: MALARIA - BLOOD STAGES

1:30 PM - 5:15 PM

Coral IV

Chairpersons: M. Aikawa and W. Weidanz

Time Abstract

1:30	15	TROPICAL MEDICINE COMMEMORATIVE FUND LECTURE. K.N. Mendis. University of Colombo, Colombo, SRI LANKA.
2:15	16	SPECIFIC SUPPRESSION OF <u>PLASMODIUM FALCIPARUM</u> GAMETOCYTEA IN IMMUNE RESIDENTS OF ARSO PIR, IRIAN JAYA. J.K. Baird*, T.R. Jones, B. Leksana, Purnomo, and S. Masbar. U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, APO San Francisco, CA.
2:30	17	INFLUENCE OF MAJOR HISTOCOMPATIBILITY COMPLEX GENES ON THE SPECIFICITY OF THE ANTIBODY RESPONSE TO THE P. <u>FALCIPARUM</u> MAJOR MEROZOITE SURFACE PROTEIN. S.P. Chang*, G.S.N. Hui, A. Kato, and W.A. Siddiqui. University of Hawaii, Honolulu, HI.

MONDAY AFTERNOON, DECEMBER 11

SCIENTIFIC SESSION A: MALARIA - BLOOD STAGES (Continued)

- 2:45 18 THE ROLE OF CONSERVED REGIONS ON THE P. FALCIPARUM MAJOR MEROZOITE SURFACE PRECURSOR PROTEIN (GP195) IN PROTECTIVE IMMUNITY. G.S.N. Hui*, L.Q. Tam, S.P. Chang, and W.A. Siddiqui. University of Hawaii, Honolulu, HI.
- 3:00 COFFEE BREAK
- 3:30 19 IDENTIFICATION OF REGIONS OF BLOOD-STAGE MALARIAL PROTEINS THAT MAY COMPRISE VACCINE COMPONENTS. A.W. Thomas*, M. Gross, D.A. Carr, J.D. Chulay, and J.A. Lyon. Walter Reed Army Institute of Research, Washington, DC; Smith Kline Beckman, King of Prussia, PA.
- 3:45 20 IMMUNIZATION TRIALS WITH THE RESA ANTIGEN OF PLASMODIUM FALCIPARUM IN AOTUS MONKEYS. W.E. Collins*, R.F. Anders, T.K. Ruebush, II, D.J. Kemp, G.H. Campbell, and G. Woodrow. Centers for Disease Control, Atlanta, GA; Walter and Elisa Hall Institute of Medical Research and Biotechnology, Melbourne, AUSTRALIA.
- 4:00 21 ANTIBODY RESPONSES OF AOTUS MONKEYS IMMUNIZED WITH SYNTHETIC PEPTIDES DERIVED FROM AMINO ACID SEQUENCES OF PLASMODIUM FALCIPARUM BLOOD-STAGE PROTEINS. G.H. Campbell*, T.K. Ruebush, II, M.E. Patarroyo, A. Moreno, W.E. Collins, R. Rodriguez, and M. Salcedo. Centers for Disease Control, Atlanta, GA and Instituto de Immunologia, Hospital San Juan de Dios, Bogota, COLOMBIA
- 4:15 22 MALARIAL PARASITES MAY USE DIFFERENT LIPID MODIFICATIONS TO TARGET PROTEINS TO SPECIFIC MEMBRANES. R.T. Reese* and H.A. Stanley. The Agouron Institute, La Jolla, CA.
- 4:30 23 USE OF A FUSION PEPTIDE AND ANTI-ID ANTIBODIES TO ASSESS THE IMMUNOGENICITY OF A PLASMODIUM YOELII SOLUBLE ANTIGEN. S. Changkasiri* and D. Taylor. Georgetown University, Washington, DC.
- 4:45 24 A NEWLY IDENTIFIED 260 KD PROTEIN OF INTRAERYTHROCYTIC PLASMODIUM FALCIPARUM PARASITES CROSS-REACTS WITH THE 11.1 PROTEIN AND PF155-RESA. C. Petersen*, R. Nelson, W. Wollish, and J. Leech. San Francisco General Hospital and University of California, San Francisco, CA.
- 5:00 25 SERUM MARKERS OF CELL MEDIATED IMMUNITY IN MALARIA. H.K. Webster*, A.E. Brown, C. Wongsrichanalai, S. Kongchareon. U.S. Army Medical Component, AFRIMS, Bangkok, THAILAND.

MONDAY AFTERNOON, DECEMBER 11

SCIENTIFIC SESSION B: VIROLOGY - DIAGNOSIS AND EPIDEMIOLOGY

DIAGNOSIS

1:30 PM - 3:00 PM

South Pacific I - II

Chairpersons: T. Tsai and F. Knauert

Time Abstract

- | | | |
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| 1:30 | 26 | A GENERAL PROCEDURE FOR PRODUCTION OF VIRAL ANTIGENS FOR ENZYME IMMUNOSORBENT ASSAY FROM INFECTED TISSUE CULTURE CELLS. T.G. Ksiazek*, F.R. Bethke, and J. Smith. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD. |
| 1:45 | 27 | APPLICATION OF ANTIGEN CAPTURE ENZYME IMMUNOASSAY FOR THE DETECTION OF WESTERN EQUINE ENCEPHALITIS VIRUS (WEE) IN MOSQUITO POOLS. P.A. Weber* and J.L. Hardy. School of Public Health, University of California at Berkeley, Berkeley, CA. |
| 2:00 | 28 | EVALUATION OF ENZYME IMMUNOASSAY FOR DIAGNOSIS OF EASTERN EQUINE ENCEPHALITIS IN EQUINES IN FLORIDA. J.G. Olson*, E.P.J. Gibbs, B. All, H.L. Rubin, and J.H. Wilson. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT; University of Florida, Gainesville, FL; and Animal Disease Diagnostic Laboratory, Kissimmee, FL. |
| 2:15 | 29 | SENSITIVITY OF IGM ELISA AND HI IN PATIENTS WITH DENGUE INFECTION CONFIRMED BY VIRUS ISOLATION. E.B. Hayes, D.G. Gubler, I.G. Gomez, and G.S. Sather. San Juan Laboratories, Centers for Disease Control, San Juan, PUERTO RICO. |
| 2:30 | 30 | DEVELOPMENT OF AN IMMUNOSORBENT-BASED FILTER HYBRIDIZATION ASSAY FOR THE DETECTION OF RIFT VALLEY FEVER VIRUS RNA. F.K. Knauert* and B.A. Parrish. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick MD. |
| 2:45 | 31 | DIAGNOSIS OF TICK-BORNE VIRUS INFECTIONS USING THE POLYMERASE CHAIN REACTION. V.K. Ward, L.D. Jones, M.A. Morse, and P.A. Nuttall*. NERC Institute of Virology and Environmental Microbiology, Oxford, UK. |
| 3:00 | | COFFEE BREAK |

MONDAY AFTERNOON, DECEMBER 11

SCIENTIFIC SESSION B: VIROLOGY - DIAGNOSIS AND EPIDEMIOLOGY (Continued)

EPIDEMIOLOGY

3:30 PM - 5:00 PM

South Pacific I - II

Chairpersons: C. Hayes and J. Childs

Time Abstract

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| 3:30 | 32 | INAPPARENT JUNIN VIRUS (JV) INFECTION AMONG RURAL MALES IN AREAS ENDEMIC FOR ARGENTINE HEMORRHAGIC FEVER (AFH). J.G. Barrera Oro*, J.I. Maiztegui, C. Saavedra, B.G. Mahlandt, S. Levis, J. Spisso, E. Tiano, M.R. Feuillade, and K.T. McKee, Jr. The Salk Institute (GSD), Frederick, MD; INEVH, Pergamino, ARGENTINA; and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD. |
| 3:45 | 33 | RISK FACTORS FOR CRIMEAN-CONGO HEMORRHAGIC FEVER IN RURAL NORTHERN SENEGAL. L.E. Chapman*, M.L. Wilson, B. LeGuanno, and S.P. Fisher-Hoch. Centers for Disease Control, Atlanta, GA; Institut Pasteur, Dakar, SENEGAL; and U.S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD. |
| 4:00 | 34 | EXAMINATION OF SERA COLLECTED BY THE HEMORRHAGIC FEVER COMMISSION DURING THE KOREAN CONFLICT FOR ANTIBODIES TO HANTAVIRUSES. J.W. LeDuc*, T.G. Ksiazek, and C.A. Rossi. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD. |
| 4:15 | 35 | RAT AND MOUSE CONTACT AND INFECTION WITH TWO RODENT-BORNE VIRUSES IN AN URBAN POPULATION FROM BALTIMORE, MD. J.E. Childs*, G.E. Glass, T.G. Ksiazek, C.A. Rossi, and J.W. LeDuc. Johns Hopkins University, Baltimore, MD and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD. |
| 4:30 | 36 | PROSPECTIVE EPIDEMIOLOGICAL STUDY ON DENGUE INFECTION OF U.S. PEACE CROPS VOLUNTEERS STATIONED IN THE PHILIPPINES. C.G. Hayes*, T.F. O'Rourke, and A.R. Sarr. U.S. Naval Medical Research Unit and U.S. Peace Corps, Manila, REPUBLIC OF THE PHILIPPINES. |
| 4:45 | 37 | SENSITIVITY AND SPECIFICITY OF CLINICAL CASE DEFINITIONS FOR DENGUE FEVER. S.H. Waterman*, D.J. Gubler, G.E. Sather, and R. Bailey. Los Angeles County Department of Health Services, Los Angeles, CA and Centers for Disease Control, San Juan, PUERTO RICO. |

MONDAY AFTERNOON, DECEMBER 11

SCIENTIFIC SESSION C: FILARIASIS - IMMUNOREGULATION

1:30 PM - 4:30 PM

South Pacific III - IV

Chairpersons: J.W. Kazura and D.O. Freedman

<u>Time</u>	<u>Abstract</u>	
1:30	38	MICE HOMOZYGOUS FOR THE MUTATION 'SEVERE COMBINED IMMUNODEFICIENCY' (SCID) SUPPORT THE GROWTH, MATURATION AND DEVELOPMENT OF INFECTIVE LARVAE OF THE HUMAN FILARIAL PARASITE <u>BRUGIA MALAYI</u> . T.V. Rajan*, D.L. Greiner, and L.D. Schultz. University of Connecticut Health Center, Farmington, CT and Jackson Laboratory, Bar Harbor, ME.
1:45	39	CYTOKINE REGULATION OF IgE PRODUCTION IN HUMAN FILARIASIS. C.L. King*, E.A. Ottesen, and T.B. Nutman. National Institutes of Health, Bethesda, MD.
2:00	40	REGULATION OF HELMINTH-INDUCED EOSINOPHILIA: INDUCTION OF INTERLEUKIN-5 mRNA. A.P. Limaye*, E.A. Ottesen, and T.B. Nutman. National Institutes of Health, Bethesda, MD.
2:15	41	HUMAN FILARIAL PARASITES SYNTHESIZE PROSTAGLANDINS. L.X. Liu*, and P.F. Weller. Beth Israel Hospital, Harvard Medical School, Boston, MA.
2:30	42	HOST RESPONSES TO <u>BRUGIA PAHANGI</u> INFECTION IN JIRDS BORN TO <u>BRUGIA</u> INFECTED MOTHERS. S.C. Bosshardt*, C.S. McVay, S.U. Coleman, and T.R. Klei. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.
2:45	43	IN VITRO B AND T CELL RESPONSIVENESS TO RECOMBINANT FILARIAL ANTIGENS. D.L. Ellenberger*, N.J. Pieniazek, M.L. Eberhard, and P.J. Lammie. Louisiana State University Medical Center, New Orleans, LA; Emory University, Atlanta, GA; and Centers for Disease Control, Atlanta, GA.
3:00		COFFEE BREAK
3:30	44	MOLECULAR DEFINITION OF A T CELL EPITOPE IN HUMAN LYMPHATIC FILARIASIS. T.B. Nutman, P. Arasu, N. Raghavan, D.O. Freedman, V. Kumaraswami, K. Jayaraman, and F.B. Perler. National Institutes of Health, Bethesda, MD; New England Biolabs, Beverly, MA; and Anna University, Madras, INDIA.
3:45	45	IgG3 ANTIBODY REACTIVITY TO A CLONED <u>BRUGIA</u> ANTIGEN CORRELATES WITH AMICROFILAREMIC DISEASE-FREE STATUS IN HUMANS WITH LYMPHATIC FILARIASIS. J.W. Kazura*, P. Maroney, K. Forsyth, M. Alpers, and T. Nilsen. Case Western Reserve University, Cleveland, OH and Papua New Guinea Institute of Medical Research, Goroka, NEW GUINEA.

MONDAY AFTERNOON, DECEMBER 11

SCIENTIFIC SESSION C: FILARIASIS - IMMUNOREGULATION (Continued)

- 4:00 46 ANALYSIS OF ISOTYPE-SPECIFIC ANTI-FILARIAL ANTIBODIES IN A HAITIAN PEDIATRIC POPULATION. W.L. Hitch*, P.J. Lammie, and M.L. Eberhard. Louisiana State University Medical Center, New Orleans, LA; Centers for Disease Control, Atlanta, GA.
- 4:15 47 CIRCULATING PARASITE ANTIGEN IN BRUGIA PAHANGI-INFECTED JIRDS. G.J. Weil*, R. Chandrashekar, F. Ifeifis, C.S. McVay, S.C. Bosshardt, and T.R. Klei. Washington University, St. Louis, MO and Louisiana State University, Baton Rouge, LA.

5th ANNUAL MEETING OF THE AMERICAN COMMITTEE ON MEDICAL ENTOMOLOGY

1:30 PM - 6:00 PM

Nautilus II - III

SYMPOSIUM: DEVELOPMENTAL FINE STRUCTURE OF ETIOLOGIC AGENTS IN VECTOR ARTHROPODS

Chairperson: A. R. Barr

- | Time | Abstract |
|------|--|
| 1:30 | 48 INTRODUCTION. A.R. Barr. School of Public Health, University of California, Los Angeles, CA. |
| 1:45 | 49 INFECTION AND MORPHOGENESIS OF ARBOVIRUSES IN MOSQUITO MESENTERONAL EPITHELIAL CELLS. E.J. Houk. School of Public Health, University of California, Los Angeles, CA. |
| 2:15 | 50 AN INSIDE LOOK AT VECTOR COMPETENCE: IN SITU STUDIES OF RIFT VALLEY FEVER VIRUS IN MOSQUITOES. W.S. Romoser. Ohio University, Athens, OH. |
| 2:45 | 51 FINE STRUCTURE OF PARASITIC AND SYMBIOTIC ORGANISMS DEVELOPING IN FLEAS. A.F. Hlad and C.B. Beard. University of Maryland School of Medicine, Baltimore, MD, and Yale University, New Haven, CT. |
| 3:15 | COFFEE BREAK |
| 3:30 | 52 DEVELOPMENT OF THE LYME DISEASE SPIROCHELE IN <u>IXODES</u> TICKS. A. Spielman. Harvard School of Public Health, Harvard University, Boston, MA. |
| 4:00 | 53 DEVELOPMENT OF <u>LEISHMANIA</u> IN PHLEBOTOMINE SANDFLIES. L.L. Walters. Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK. |
| 4:30 | 54 DEVELOPMENT OF MALARIA PARASITES IN MOSQUITOES. J.P. Vanderberg. New York University School of Medicine, New York, NY. |
| 5:00 | PRESENTATION OF THE HOOGSTRAAL MEDAL. |
| 5:30 | BUSINESS MEETING OF AMERICAN COMMITTEE OF MEDICAL ENTOMOLOGY. |

MONDAY AFTERNOON, DECEMBER 11

SEMINAR ON THE LEGISLATIVE PROCESS: ADVOCACY WORKSHOP*

5:00 PM - 6:00 PM

South Pacific III - IV

Chairpersons: J.K. Frenkel and S.R. Sagebiel

SUMMARY: This seminar will provide a course of instruction on the legislative process, with handouts and reference materials. It will be given by professional lobbyists and Congressional staff.

PRESENTERS: Pat DeLeon Office of Senator Inouye
Margaret Donahue Capitol Associates, Inc.
Congressional Staff Office of Representative Waxman
(To be announced)

I. THE LEGISLATIVE PROCESS: FROM CONCEPT TO ENACTMENT

- A. AUTHORIZATION:
 - 1. Purpose, Rationale and Hearings
- B. BUDGETING:
 - 1. Budget Deficit Reduction Act
- C. APPROPRIATIONS:
 - 1. Purpose, Rationale, Cycle
 - 2. Agency Testimony
 - 3. Public Witness Testimony
 - 4. Report and Bill Language

II. ROLES OF FEDERAL AGENCIES IN THE LEGISLATIVE PROCESS

- A. DEPARTMENT CLEARANCE
(e.g., Health and Human Services, Defense, and State)
- B. OMB REVIEW AND CLEARANCE

III. ROLES OF SPECIAL INTEREST GROUPS

- A. PUBLIC WITNESS TESTIMONY
- B. DEVELOPING RELATIONSHIPS WITH CONGRESSIONAL DELEGATIONS
- C. LETTER-WRITING CAMPAIGNS
- D. TIMING OF ADVOCACY EFFORTS

IV. FOLLOW-UP

* - This seminar is co-sponsored by the ASTMH Committee for Public Affairs, the ASTMH Legislative Task Force and Capitol Associates, Inc.

MONDAY AFTERNOON, DECEMBER 11

OPEN ORGANIZATIONAL MEETING: AMERICAN COMMITTEE ON MEDICAL MALACOLGY

5:00 PM

Sea Pearl II - III

Chairperson: W.A. Sodeman

TUESDAY MORNING, DECEMBER 12

POSTER SESSION I WITH CONTINENTAL BREAKFAST

7:30 - 10:30 AM

Coral Lounge

AUTHORS IN ATTENDANCE FROM 8:00 AM - 10:00 AM

The poster boards will be available in the Coral Lounge beginning at 7:00 PM Monday evening. Posters should be set up by 8:00 AM Tuesday morning and taken down by 5:00 PM Tuesday afternoon.

FILARIASIS

- 55 MONOCLONAL ANTIBODIES REACTIVE WITH SURFACE ANTIGENS ON INFECTIVE LARVAE OF ONCHOCERCA SPP. AND DIROFILARIA IMMITIS DO NOT REACT WITH SURFACE ANTIGENS OF BRUGIA MALAYI OR ACANTHOCEILONEMA VITEAE. R.B. Grieve*, M. Mika-Grieve, E.W. Cupp, G.R. Frank, M. Mok, D. Abraham, and M. Karam. Colorado State University, Fort Collins, CO; University of Arizona, Tucson, AZ; Thomas Jefferson University, Philadelphia, PA; and the Onchocerciasis Control Programme, Ouagadougou, BURKINA-FASO.
- 56 STUDIES ON THE REGULATION OF EXPRESSION OF A 16KD SURFACE-ASSOCIATED ANTIGEN OF BRUGIA MALAYI. N. Storey* and M. Philipp. Molecular Parasitology Group, New England Biolabs, Beverly, MA.
- 57 BRUGIA MALAYI: CHARACTERIZATION OF LAMBDA gt11 CLONE EXPRESSING A POTENTIALLY DIAGNOSTIC ANTIGEN. S. Dissanayake* and W.F. Piessens. Harvard School of Public Health, Boston, MA.

MONDAY AFTERNOON, DECEMBER 11

POSTER SESSION I (FILARIASIS - Continued)

- 58 BRUGIA MALAYI: ANTIBODIES TO AN ONCHOCERCA VOLVULUS HEAT SHOCK PROTEIN 70 IN AMICROFILAREMIC JIRDS. J. Yates*, K. Schmitz, N. Rothstein, and T.V. Rajan. Oakland University, Rochester, MI and University of Connecticut Health Center, Farmington, CT.
- 59 VARIATIONS IN COMPLEMENT MEDIATED CELLULAR ADHERENCE AND CYTOTOXICITY TO MICROFILARIAE OF BRUGIA PATEI, BRUGIA MALAYI AND BRUGIA PHAHANGI. U.R. Rao, B.H. Kwa*, and A.C. Vickery. College of Public Health, University of South Florida, Tampa, FL.
- 60 ULTRASTRUCTURAL CHANGES IN THE LYMPH NODES OF BRUGIA MALAYI INFECTED NUDE MICE PRIOR TO THE ONSET OF LYMPHATIC DILATION. E. Toro, B.H. Kwa, and A.C. Vickery*. University of South Florida, Tampa, FL.
- 61 HUMAN IMMUNE RESPONSES TO ONCHOCERCA VOLVULUS: POSSIBLE RELATIONSHIP BETWEEN SPECIFIC ISOTYPIC ACTIVITIES TO ANTIGENS AND STATE OF IMMUNITY. A.E. Boyer*, V.C.W. Tsang, M.L. Eberhard, J.A. Brand, W. Zhou, and L. Laughnan. Centers for Infectious Disease, Centers for Disease Control, Atlanta, GA.
- 62 IMMUNOLOGICAL ACTIVATION ASSOCIATED WITH IVERMECTIN-INDUCED MICROFILARIAL CLEARANCE. P. Lammie*, M. Eberhard, R. Bryan, F. Richards, D. McNecley, and H. Spencer. Tulane University, New Orleans, LA; Centers for Disease Control, Atlanta, GA; and St. Croix Hospital, Leogane, HAITI.
- 63 ISOLATION OF A HYPERVARIABLE REPETITIVE DNA ELEMENT (VNTR) FROM THE GENOME OF THE HUMAN FILARIAL PARASITE, WUCHERERIA BANCROFTI. S. Natarajan* and T.V. Rajan. University of Connecticut Health Center, Farmington, CT.
- 64 A FILARIAL ANTIGEN RECOGNIZED BY MANY PATIENTS WITH HUMAN LYMPHATIC FILARIASIS BEARS STRONG SEQUENCE HOMOLOGY TO MAMMALIAN TYPE 4 (BASEMENT MEMBRANE) COLLAGEN. C.R. Vanamala*, C. Werner, and T.V. Rajan. University of Connecticut Health Center, Farmington, CT, and Albert Einstein College of Medicine, Bronx, NY.
- 65 ISOLATION AND CHARACTERIZATION OF WUCHERERIA BANCROFTI RECOMBINANT CLONES RECOGNIZED BY ANTISERA TO A POTENTIALLY PROTECTIVE 43 KD ANTIGEN OF BRUGIA MALAYI INFECTIVE LARVAE. D.O. Freedman*, N. Raghavan, T.B. Nutman, and E.A. Ottesen. National Institute of Allergy and Infectious Diseases, Bethesda, MD.
- 66 MAPPING THE B CELL EPITOPES OF FILARIAL PARAMYOSIN WITH SERA FROM PATIENTS WITH ONCHOCERCIASIS. C. Steel*, R. Limberger, T.B. Nutman, C. Maina, and L.A. McReynolds. National Institutes of Health, Bethesda, MD and New England Biolabs, Beverly, MA.
- 67 SUSCEPTIBILITY OF IMMUNODEFICIENT MOUSE STRAINS TO INFECTION WITH LARVAE OF BRUGIA MALAYI. C.J. Prain and M. Philipp. New England Biolabs, Beverly, MA.

MONDAY AFTERNOON, DECEMBER 11

POSTER SESSION I (FILARIASIS - Continued)

- 68 AN ONCHOCERCA VOLVULUS ANTIGEN WHICH IS PREFERENTIALLY RECOGNIZED BY SERA FROM 'PUTATIVELY IMMUNE' ENDEMIC. S.E. Roemer*, T.B. Nutman, M.W. Southworth, and F.B. Perler. New England Biolabs, Beverly, MA and National Institutes of Health, Bethesda, MD.
- 69 ELEVATED TISSUE EOSINOPHILIA AND SERUM IgG IN EXPERIMENTAL MURINE OCULAR ONCHOCERCIASIS. E.R. James, A. Hodgson-Smith, M. Jackson-Gegan, B. Smith, D. McLean, H.L. Callahan, and J. Renfro, III. Medical University of South Carolina, Charleston, SC.
- 70 IDENTIFICATION OF RODENT HOSTS FOR LARVAL STAGES OF ONCHOCERCA LIENALIS. D. Abraham*, J.B. Lok, A.J. Ripepi, and A.M. Lange. Thomas Jefferson University, Philadelphia, PA and University of Pennsylvania, Philadelphia, PA.
- 71 LARGE SCALE IN VITRO PRODUCTION OF ONCHOCERCA VOLVULUS FOURTH STAGE LARVAE. B. Brotman, A.B. Smith, and A.M. Prince. The Lindsley F. Kimball Research Institute of the New York Blood Center, New York, NY and Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, LIBERIA.
- 72 BRUGIA PAHANGI IN DOGS: BASIC PARASITOLOGICAL DATA. J.W. McCall*, M.D. Dzimianski, J.J. Jun, and P. Supakorndej. University of Georgia, Athens, GA.
- 73 GRANULOMATOUS REACTIVITY OF FRACTIONATED BRUGIA PAHANGI ADULT ANTIGENS IN HOMOLOGOUSLY INFECTED JIRDS. R.G. Farrar, T.R. Klei*, M.D. West, R.C. Montelaro, C.S. McVay, and S.U. Coleman. Louisiana State University, Baton Rouge, LA.
- 74 CHARACTERIZATION OF A GENOMIC CLONE FROM ONCHOCERCA VOLVULUS HOMOLOGOUS TO A BRUGIA MALAYI GENE FOR A POTENTIALLY PROTECTIVE ANTIGEN. M.A. Kron*, K. Erttmann, T. Unnasch, and B. Greene. Case Western Reserve University, Cleveland, OH and University of Alabama at Birmingham, Birmingham, AL.
- 75 PHOSPHOCHOLINE CONTAINING ANTIGENS OF BRUGIA MALAYI NON-SPECIFICITY SUPPRESS LYMPHOCYTE FUNCTION. R.B. Lal*, V. Kumaraswami, C. Steel, and T.B. Nutman. Uniformed Services University of the Health Sciences, Bethesda, MD; National Institutes of Health, Bethesda, MD, and Tuberculosis Research Institute, Madras, INDIA.
- 76 ACTIVATION OF JIRD B AND T LYMPHOCYTES BY RECOMBINANT FILARIAL ANTIGENS. N.J. Pieniazek*, D.L. Ellenberger, and P.J. Lammie. Centers for Disease Control, Atlanta, GA; Louisiana State University Medical Center, New Orleans, LA; and Emory University, Atlanta, GA.

MALARIA - PRE-ERYTHROCYTIC STAGES

- 77 INTRACYTOPLASMIC CIRCUMSPOROZOITE PROTEIN IS TRANSLOCATED TO THE SURFACE OF GLIDING MALARIA SPOROZOITES AT THEIR ANTERIOR ENDS. M.J. Stewart* and J.P. Vanderberg. New York University School of Medicine, New York, NY.

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POSTER SESSION I (MALARIA - PRE-ERYTHROCYTIC STAGES - Continued)

- 78 DETECTION OF THE CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM FALCIPARUM IN MOSQUITO TISSUES. C.F. Golenda, I. Schneider, and R.A. Wirtz. Walter Reed Army Institute of Research, Washington, DC.
- 79 A RECOMBINANT P. FALCIPARUM CIRCUMSPOROZOITE (CS) PROTEIN DESIGNED WITH A HYDROPHOBIC DECAPEPTIDE ANCHOR (R32FT) INCREASES IMMUNOGENICITY IN CS-REPEAT RESPONDER AND NON-RESPONDER MICE WHEN HYDROPHOBICALLY COMPLEXED TO PROTEOSOMES. R. Jaffe*, L. Loomis, W. Zollinger, M. Monheit, W. Ballou, A. Fisher, M. Gross, J. Young, J. Sadoff, and G. Lcwell. Walter Reed Army Institute of Research, Washington, DC. and Smith Kline and French Laboratories, Philadelphia, PA.
- 80 FINE SPECIFICITY OF POLYCLONAL SERA RAISED AGAINST THE NON-REPEATING REGIONS OF THE PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN. M.C. Seguin*, M.R. Hollingdale, C. Silverman, G.F. Wasserman, M. Gross, and D.M. Gordon. Walter Reed Army Institute of Research, Washington, DC; Biomedical Research Institute, Rockville, MD; and Smith Kline and French Laboratories, Swedeland, PA.
- 81 IN VITRO INHIBITION OF EXO-ERYTHROCYTIC SCHIZONT DEVELOPMENT BY SERA FROM SAIMIRI MONKEYS IMMUNIZED WITH PLASMODIUM VIVAX RECOMBINANT CIRCUMSPOROZOITE PROTEINS. P. Millet*, R. Rosenberg, W.E. Collins, R. Wirtz, and J.R. Broderon. Centers for Disease Control, Atlanta, GA; AFRIMS Bangkok, THAILAND; and Walter Reed Army Institute of Research, Washington, DC.
- 82 SAFETY AND IMMUNOGENICITY OF RECOMBINANT PLASMODIUM VIVAX SPOOROZOITE VACCINE IN VOLUNTEERS. D. Herrington*, E.J. Nardin, R. Bank, G.A. Losonsky, I. Bathurst, P.J. Barr, R.S. Nussenzweig, V. Nussenzweig, and M.M. Levine. University of Maryland, Baltimore, MD; New York University Medical Center, New York, NY; and Chiron Corporation, Emeryville, CA.
- 83 A CIRCUMSPOROZOITE-LIKE PROTEIN IS PRESENT IN MICRONEMES OF MATURE BLOOD STAGES OF MALARIA PARASITES. A.H. Cochrane*, S. Uni, M. Maracic, L. di Giovanni, M. Aikawa, and R.S. Nussenzweig. New York University School of Medicine, New York, NY and Case Western Reserve University, Cleveland, OH.
- 84 A SIMPLE DOT-IMMUNOBINDING TECHNIQUE FOR THE DETECTION OF PLASMODIUM FALCIPARUM SPOOROZOITES IN MOSQUITOS. G.W. Long and J.J. Oprandy. Naval Medical Research Institute, Bethesda, MD.
- 85 DESIGNING PROTEOSOME-PEPTIDE VACCINES TO INDUCE ANTIBODIES AGAINST THE HIGHLY CONSERVED PENTAPEPTIDE N1a (KLKQP) OF MALARIA CS PROTEINS: EFFECTS OF REPLICATING THE HYDROPHOBIC ANCHOR AND/OR N1a. G.H. Lowell, L.F. Smith, M. Carter, S. Aley, R. Jaffe, R. Wirtz, P. Leland, V. Harrod, C. Schultz, W.D. Zollinger, D. Gordon, J.C. Sadoff, J. Chulay, and M.R. Hollingdale. Walter Reed Army Institute of Research, Washington, DC and Biomedical Research Institute, Rockville, MD.

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- 86 LOCALIZATION OF CS AND NON-CS ANTIGENS WITHIN PLASMODIUM FALCIPARUM EE PARASITES BY IMMUNOELECTRON MICROSCOPY. J.F.G.M. Meis, T. Ponnudurai, B. Mons, A. van Belkum, P. van Eerd, H. Schellekens, S.B. Aley, and M.R. Hollingdale. Universities of Nijmegen and Leiden Medical Schools, Primate Center TNO, Rijswijk, THE NETHERLANDS; Biomedical Research Institute, Rockville, MD.
- 87 DIAGNOSIS OF T-CELL DETERMINANTS IN PLASMODIUM FALCIPARUM BY OLIGONUCLEOTIDE: DNA HYBRIDIZATION. A.A. Lal, V.E. do Rosario, K. Sakhuja, V. de la Cruz, M.R. Hollingdale, and T.F. McCutchan. Biomedical Research Institute, Rockville, MD; National Institutes of Health, Bethesda, MD.
- 88 PLASMODIUM GALLINACEUM: INHIBITION OF SPOROZOITE INVASION (ISI) AND EE DEVELOPMENT. E.M.M. da Rocha, A. Krettli, R. Gwadz, A. Appiah, M. Murphy, and M.R. Hollingdale. Biomedical Research Institute, Rockville, MD; Fiocruz, Belo Horizonte, BRAZIL; National Institutes of Health, Bethesda, MD.
- 89 IDENTIFICATION AND PURIFICATION OF A PLASMODIUM FALCIPARUM ANTIGEN PRESENT IN SPOROZOITES, EE PARASITES AND MEROZOITES. G. Chen, M.R. Hollingdale, S.B. Aley, P. Leland, and D. Taylor. Biomedical Research Institute, Rockville, MD; Georgetown University, Washington, DC.
- 90 PLASMODIUM BERGHEI EXOERYTHROCYTIC ANTIGENS: SEROLOGY, CLONING AND GENE ANALYSIS. K. Sakhuja, B. Sina, J. Zhu, and M.R. Hollingdale. Biomedical Research Institute, Rockville, MD.
- 91 HUMORAL IMMUNE RESPONSE TO THE CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM VIVAX IN RESIDENTS OF SEVERAL VILLAGES IN THE PERUVIAN JUNGLE. E.D. Franke*, C. Lucas, H. Covenas, and E. San Roman. U.S. Naval Medical Research Institute Detachment, Lima, PERU and Occidental Petroleum Corporation of PERU.
- 92 INCREASED IMMUNOGENICITY OF A RECOMBINANT PLASMODIUM FALCIPARUM SPOROZOITE VACCINE, R32NS181, WHEN INCORPORATED INTO A LIPOSOME. J.E. Egan*, C.R. Alving, R.L. Richards, G.F. Wasserman, and C.C. Silverman. Walter Reed Army Institute of Research, Washington, DC and Smith Kline and French Laboratories, Swedeland, PA.

KINETOPLASTIDIA - IMMUNOLOGY, EPIDEMIOLOGY AND PHARMACOLOGY

- 93 BIOCHEMICALLY SIMILAR LEISHMANIA-LIKE PARASITES FROM COLOMBIA AND PANAMA. R.D. Kreutzer*, M. Grogil, D.G. Young, E.D. Rowton, R.B. Tesh, G. Grimaldi, Jr., and A. Corredor. Youngstown State University, Youngstown, OH; Walter Reed Army Institute of Research, Washington, DC; University of Florida, Gainesville, FL; Yale University School of Medicine, New Haven, CT; Instituto Oswaldo Cruz, Rio de Janeiro, BRAZIL; and Instituto Nacional de Salud, Bogota, COLOMBIA.

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POSTER SESSION I (KINETOPLASTIDIA - IMMUNOLOGY, EPIDEMIOLOGY AND
PHARMACOLOGY - Continued)

- 94 LEISHMANIA INFANTUM SENSU LATO ISOLATED FROM A GIANT RAT (CRICETOMYS GAMBIANUS) CAPTURED AT A CASE SITE OF HUMAN CUTANEOUS LEISHMANIASIS IN KENYA. P. Lawyer*, Y. Mebrahtu, P. Ngumbi, C. Angili, and J. Nzovu. Kenya Medical Research Institute and U.S. Army Medical Research Unit, Nairobi, KENYA.
- 95 A RURAL FOCUS OF HUMAN CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA TROPICA IN KENYA. Y. Mebrahtu*, P. Lawyer, G. Kirigi, and C. Roberts. Kenya Medical Research Institute, U.S. Army Medical Research Unit, and University of Nairobi Medical Faculty, Nairobi, KENYA.
- 96 ISOLATION OF LEISHMANIA TROPICA FROM R. RATTUS IN MALTA. B.C. Walton*, R. Killick-Kendrick, L. Gradoni, M. Gramiccia and D.V. Briffa. Gettysburg, PA; Imperial College, Silwood Park, UK, Istituto Superiore di Sanita, Rome, ITALY; Boffa Hospital, Floriana, MALTA.
- 97 THE DEVELOPMENT OF A SUBSPECIES-SPECIFIC RECOMBINANT DNA PROBE FOR THE CHARACTERIZATION OF LEISHMANIA MEXICANA MEXICANA (LMX). J.M. Stiteler*, P.V. Perkins, E.D. Rowton, and L.W. Roberts. Walter Reed Army Institute of Research, Washington, DC.
- 98 HUMAN T CELL CLONES SPECIFIC FOR DOMINANT LEISHMANIA ANTIGENS. S.G. Reed*, E.M. Carvalho, C.H. Sherbert, K.H. Grabstein, and W.D. Johnson, Jr. Seattle Biomedical Research Institute, Seattle, WA; University of Bahia, Salvador, BRAZIL; Immunex Corporation, Seattle, WA; and Cornell Medical College, New York, NY.
- 99 AN ENZYME IMMUNOSORBENT ASSAY (EIA) FOR THE SENSITIVE AND SPECIFIC DIAGNOSIS OF CHAGAS' DISEASE: THE USE OF AMASTIGOTE AND EPIMASTIGOTE ANTIGENS. A.A. Pan*, G.B. Rosenberg, M. Hurley, G. Schock, V. Chu, and A. Aiyappa. Abbott Laboratories, Abbott Park, IL.
- 100 ANTI-INTERFERON ANTIBODIES WITH DIVERSE SPECIFICITIES MODULATE NATURAL RESISTANCE OF C3H/HeN MICE DIFFERENTLY DURING THE FIRST WEEK OF INFECTION WITH LEISHMANIA MAJOR. D.A. Leiby*, D.S. Finbloom, R.D. Schreiber, and C.A. Nacy. Walter Reed Army Institute of Research, Washington, DC; Food and Drug Administration, Bethesda, MD; and Washington University, St. Louis, MO.
- 101 LOCALIZED CUTANEOUS LEISHMANIASIS (CHICLERO ULCER) IN MEXICO; SENSITIVITY AND SPECIFICITY OF ELISA FOR IgG ANTIBODIES TO LEISHMANIA MEXICANA MEXICANA. M.R. Garcia*, F. Andrade, R. Esquivel, E. Simmonds, S. Canto, and A.L. Cruz. University of Yucatan (Mexico) Tropical Diseases Research Unit; Reference Center for Leishmaniasis Control. ??

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POSTER SESSION I (KINETOPLASTIDIA - IMMUNOLOGY, EPIDEMIOLOGY AND
PHARMACOLOGY - Continued)

- 102 SERODIAGNOSIS OF HUMAN AND CANINE LEISHMANIASIS BY A RADIOIMMUNOASSAY. M.V. Londner*, A.G.E. Missari, G. Rosen, T.A. Morsy, S.M. Afram, S. El Said, and S.H. Giannini. The Kuvshin Centre, Hebrew University-Hadassah Medical School, Jerusalem, ISRAEL; Ain Shams University Research and Training Center on Vectors of Diseases, Cairo, EGYPT; and University of Maryland School of Medicine, Baltimore, MD.
- 103 A RAPID AND RELIABLE SERODIAGNOSTIC TEST FOR ACUTE VISCERAL LEISHMANIASIS. J.M. Scott*, W.G. Shreffler, R. Badaro, and S.G. Reed. Seattle Biomedical Research Institute, Seattle, WA; Federal University of Bahia, Salvador, BRAZIL.
- 104 COMPARISON OF KETOCONAZOLE TO PENTOSTAM AND TO PLACEBO IN THE TREATMENT OF LEISHMANIA BRAZILIENSIS PANAMENSIS CUTANEOUS LEISHMANIASIS: A FINAL REPORT. R.E. Saenz, H. Paz, and I.D. Bermis*. Gorgas Memorial Lab, PANAMA; Walter Reed Army Institute of Research, Washington, DC.
- 105 CLINICAL RELEVANCE OF IN VITRO ANTILEISHMANIAL SUSCEPTIBILITY TESTING. M. Grogl*, T.N. Thomason, D. Panisko, J.S. Keystone, W.A. Reid, and D.E. Kyle. Walter Reed Army Institute of Research, Washington, DC and Toronto General Hospital, Toronto, Ontario, CANADA.
- 106 COMPARATIVE ANALYSIS OF DIFFERING SENSITIVITIES TO PENTOSTAM OF TWO SPECIES OF LEISHMANIA BY PULSE-FIELD GEL ELECTROPHORESIS. R.K. Martin*, M. Grogl, N.A. Edwards, and D.E. Kyle. Walter Reed Army Institute of Research, Washington, DC.
- 107 QUANTITATIVE IN VITRO DRUG SUSCEPTIBILITY TESTS OF LEISHMANIA SPP FROM PATIENTS UNRESPONSIVE TO PENTAVALENT ANTIMONY (SBV) THERAPY. J.E. Jackson*, J.D. Tally, Y.B. Mebrahtu, P.G. Lawyer, J.B.C. Were, and S.G. Reed. Walter Reed Army Institute of Research, Washington, DC, U.S. Army Medical Research Unit, Nairobi, KENYA; and Seattle Biomedical Research Institute, Seattle, WA.
- 108 IN VITRO QUANTIFICATION OF LOT-TO-LOT VARIABILITY IN ANTILEISHMANIAL ACTIVITY OF PENTAVALENT ANTIMONIALS. J.E. Jackson*, J.D. Tally, and W.Y. Ellis. Walter Reed Army Institute of Research, Washington, DC.

SCHISTOSOMIASIS

- 109 CHARACTERIZATION OF A SCHISTOSOMA MANSONI FEMALE-SPECIFIC GENE THAT PUTATIVELY ENCODES A 48 KD EGG-SHELL PROTEIN. L. Chen*, H. Hirai, D.M. Rekosh, and P.T. LoVerde. State University of New York, Buffalo, NY.
- 110 SCHISTOSOMA MANSONI LARVICIDAL ACTIVITY OF MURINE BRONCHOALVEOLAR LAVAGE CELLS. F.A. Lewis*, C.A. White, J.E. Bill, and G.M. Niemann. Biomedical Research Institute, Rockville, MD.

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POSTER SESSION I (SCHISTOSOMIASIS - Continued)

- 111 IMMUNIZATION OF MICE WITH THE MAJOR LYMPHOPROLIFERATIVE PEAK OF FRACTIONATED SOLUBLE EGG ANTIGEN (SEA) INDUCES A HUMORAL RESPONSE TO BOTH CARBOHYDRATE AND POLYPEPTIDE EPITOPES. J.J. Quinn and D.A. Harn. Harvard School of Public Health, Boston, MA.
- 112 TRANSMISSION OF LYMPHOCYTE RESPONSIVENESS TO SCHISTOSOMAL ANTIGENS BY BREAST FEEDING. A.M. Eissa, M.A. Saad, A.K. Abdel Ghaffar, I.M. El-Sharkawy and K.A. Kamal*. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT and Medical School, Al-Azhar University, Cairo, EGYPT.
- 113 SCHISTOSOMA MANSONI INFECTION REDUCES THE PERCENT OF VIABLE LITTERS FROM PREGNANT CBA MICE AND ALTERS IMMUNE RESPONSIVENESS OF THE OFFSPRING. T. Amano*, G.L. Freeman, Jr., and D.G. Colley. Veterans Administration Medical Center and Vanderbilt University School of Medicine, Nashville, TN.
- 114 PERCUTANEOUS COMPARED TO PER-ORAL SCHISTOSOMA MANSONI INFECTIONS IN HAMSTERS. P.G. Kremsner, S. Mravak, and G. Poggensee. State Institute of Tropical Medicine, Berlin, WEST GERMANY.
- 115 DEVELOPMENTALLY REGULATED EXPRESSION OF SCHISTOSOMA MANSONI GLYCOPROTEINS. B. Koster and M. Strand. Johns Hopkins University School of Medicine, Baltimore, MD.
- 116 THE IMMUNE-DEPENDENT ACTION OF PRAZIQUANTEL: MOLECULAR CHARACTERIZATION OF A SCHISTOSOMA MANSONI TARGET ANTIGEN. T.M. Tanaka* and M. Strand. Johns Hopkins University School of Medicine, Baltimore, MD.
- 117 ANTI ELASTASE ANTIBODIES AS SERO-EPIDEMIOLOGICAL INDICATORS OF EXPOSURE TO TRANSMISSION OF SCHISTOSOMIASIS. C.J. Shiff, A. Esfandiari, G. Kinoti, and J. McKerrow. Johns Hopkins University, Baltimore MD; Post-Graduate Medical School, Los Angeles, CA; University of Nairobi, Nairobi, KENYA; and University of California at San Francisco, San Francisco, CA.

OTHER HELMINTHS

- 118 EOSINOPHILIC MENINGOENCEPHALITIS DUE TO ANGIOSTRONGYLUS CANTONENSIS AS THE CAUSE OF DEATH IN CAPTIVE NONHUMAN PRIMATES. C.H. Gardiner*, S. Wells, A.E. Gutter, L. Fitzgerald, D.C. Anderson, R.K. Harris, and D.K. Nichols. Armed Forces Institute of Pathology, Washington, DC; Audubon Park and Zoological Gardens, New Orleans, LA; Ardastra Gardens and Zoo, Nassau, BAHAMAS; Yerkes Regional Primate Research Center, Emory University, Atlanta, GA; and Zoo/Path, Bowie, MD.
- 119 ADULT MALE CAPILLARIID INFECTING THE ORAL MUCOSA OF MAN. R.C. Neafie* and A.J. Strano. Armed Forces Institute of Pathology, Washington, DC and St. John's Hospital, Springfield, IL.

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POSTER SESSION I (OTHER HELMINTHS - Continued)

- 120 MECHANISTIC STUDIES IN THE TRANSCUTICULAR DELIVERY OF ANTIPARASITIC DRUGS: BIOPHYSICAL TRANSPORT PROPERTIES OF THE CUTICLE OF THE PARASITIC NEMATODE ASCARIS SUUM. D.P. Thompson*, N.F.H. Ho, T.G. Geary, T.J. Raub, and C.L. Barsuhn. The Upjohn Company, Kalamazoo, MI.
- 121 LYMPHOCYTE ACTIVATION IN VITRO BY ASCARIS SUUM CUTICULAR ANTIGENS. D.E. Hill*, R.H. Fetterer, and J.F. Urban. Helminthic Diseases Laboratory, LPSI, Agriculture Research Services, U.S. Department of Agriculture, Beltsville, MD.
- 122 SEROLOGY AS AN INDICATOR OF TAENIA SOLIUM TAPEWORM INFECTIONS IN RURAL VILLAGES OF MEXICO. S.P. Díaz-Camacho, A. Candil Ruiz, M. Beltran Uribe, and K. Willms*. Escuela de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Instituto de Investigaciones Biomedicas, National University of Mexico, Mexico City, MEXICO.
- 123 EFFECT OF ZINC DEFICIENCY ON THE ESTABLISHMENT AND REPRODUCTION OF AN INTESTINAL NEMATODE IN MICE. T. Minkus, M.E. Scott*, and K. Koski. McGill University, Montreal, Canada.
- 124 PREVALENCE AND RISK FACTORS FOR TAENIA SOLIUM TAENIASIS AND CYSTICERCOSIS IN AN ENDEMIC VILLAGE, MORELOS STATE, MEXICO. E. Sarti G., P.M. Schantz, A. Plancarte, I. Gutierrez O., V.C.W. Tsang, and A. Flisser. Direccion General de Epidemiologia, Secretaria de Salud, Mexico City; Centers for Disease Control, Atlanta, GA; Instituto de Investigaciones Biomedicas, UNAM, Mexico City, MEXICO.
- 125 NUCLEAR CHANGES OF INFECTED MUSCLE CELLS CORRELATE WITH ANTIGEN SYNTHESIS AND SECRETION BY THE LARVA OF TRICHINELLA SPIRALIS. D.D. Despommier, W.F. Symmans, T. Edelist, and S. Buck. Columbia University, New York, NY.
- 126 IMMUNODIAGNOSIS OF NEUROCYSTICERCOSIS IN SALIVA. M. Feldman*, M.A. Sandoval, A. Plancarte, and A. Flisser. Instituto de Investigaciones Biomedicas, UNAM, MEXICO; D.F. Mexico, Hospital de Especialidades, Centro Medico La Raza, Mexico, D.F., MEXICO.
- 127 INVOLVEMENT OF EXCRETORY-SECRETORY ANTIGENS FROM JUVENILE ANISAKIS SIMPLEX IN THE IMMUNE-MEDIATED ADHERENCE OF EOSINOPHILS. T.L. Deardorff*, R.E. Jones, and S.G. Kayes. U.S. Food and Drug Administration, Dauphin Island, AL, and University of South Alabama College of Medicine, Mobile. AL.
- 128 INTESTINAL CAPILLARIASIS: THE SPREAD. J.H. Cross. Uniformed Services University of the Health Sciences, Bethesda, MD.
- 129 IDENTIFICATION OF SPECIES AND STAGE-SPECIFIC ANTIGENS OF PARAGONIMUS WESTERMANI WITH MONOCLONAL ANTIBODIES. Z. Zhao*, S. Yiping, and W.F. Piessens. Nanjing Medical College, Nanjing, PEOPLES'S REPUBLIC OF CHINA and Harvard School of Public Health, Boston, MA.

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POSTER SESSION I (OTHER HELMINTHS - Continued)

- 130 ISOLATION AND CHARACTERIZATION OF MOUSE EOSINOPHIL SPECIFIC GRANULE PROTEINS FOLLOWING TOXOCARA CANIS INFECTION. F.J. Herndon* and S.G. Kayes. University of South Alabama College of Medicine, Mobile, AL.
- 131 SERINE PROTEASES FROM TWO INVASIVE ASCARIDOID NEMATODES. J. Deneris and J.A. Sakanari. University of California, San Francisco, CA.

VIRUS VACCINE DEVELOPMENT

- 132 SAFETY AND IMMUNOGENICITY OF A NEW CHIKUNGUNYA VIRUS VACCINE: DOUBLE BLIND PLACEBO-CONTROLLED HUMAN TRIAL. F.J. Malinoski*, T. Ksiazek, H. Ramsburg, H.W. Lupton, and G.F. Meadors. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 133 VENEZUELAN EQUINE ENCEPHALITIS (VEE) SPECIFIC IgG SUBCLASS RESPONSES FOLLOWING IMMUNIZATION WITH TC-83 AND C-84 VACCINE. R.J.M. Eagler*, C.J. Peters, P.B. Jahrling, M. Pedrotti, J. Mangiafico, and C. Reimer. Walter Reed Army Institute of Research, Washington, DC; U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick MD; and Centers for Disease Control, Atlanta, GA.
- 134 FOURTEEN YEARS OF EXPERIENCE WITH INACTIVATED EASTERN EQUINE ENCEPHALITIS VACCINE. N.A. Popovic*, D.D. Oland, and J.A. Mangiafico. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 135 EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF AN INACTIVATED JAPANESE ENCEPHALITIS VACCINE IN U.S. LABORATORY PERSONNEL. M.A. Ussery*, N.A. Popovic, D. Oland, and J. Magnifico. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 136 PROTECTIVE EFFICACIES IN RHESUS MONKEYS OF CANDID 1 STRAIN JUNIN VIRUS (JV) AND TACARIBE VIRUS AGAINST AEROSOL CHALLENGE WITH VIRULENT JV. R.H. Kenyon*, K. McKee, Jr., J. Barrera Oro, D. Ragland, C. Crabbs, and Y. Higgins. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 137 INACTIVATED HEPATITIS A VACCINE (CLF STRAIN) IN HEALTHY ADULT VOLUNTEERS. M. Sjogren*, C. Hoke, L. Binn, J. Sanchez, L. Lyde, E. D'Hordt, J. Boscia, and W. Bancroft. Walter Reed Army Institute of Research, Washington, DC and Smith Kline Beckman, Philadelphia, PA and BELGIUM.
- 138 LOW-DOSE INTRADERMAL RECOMBINANT HEPATITIS B VACCINE. J. Bryan*, M. Iqbal, M. Sjogren, A. Ahmed, A. Rauf, S. Nabi, B. Cox, A. Morton, J. Shuck, P. Macartly, I. Malik, P. Perine, and L. Lagters. Uniformed Services University of the Health Sciences, Bethesda, MD; Army Medical College, Rawalpindi, PAKISTAN, Walter Reed Army Institute of Research, Washington, DC.

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POSTER SESSION I (VIRUS VACCINE DEVELOPMENT - Continued)

- 139 EFFICACY OF A MASS RECOMBINANT HEPATITIS B VACCINATION PROGRAM IN PRIMARY SCHOOL CHILDREN INFECTED WITH S. MANSONI. S. Bassily*, M.F. Abdel Wahab, T. Strickland, G. Esmat, S. Narooz, N. El-Masry, N. Constantine, and E. Medhat. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT; Cairo University, Cairo, EGYPT; and University of Maryland School of Medicine, Baltimore, MD.

MOLECULAR BIOLOGY - VIRUSES

- 140 ANTIGENIC ANALYSIS OF A GLOBAL COLLECTION OF HANTAVIRUS STRAINS. Y.K. Chu, S.E. Hasty, C.S. Schmaljohn, C.A. Rossi, J.W. LeDuc, and J.M. Dalrymple. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick MD.
- 141 CLONING AND SEQUENCE ANALYSIS OF THE L GENOME SEGMENTS OF TWO VIRUSES IN THE HANTAVIRUS GENUS OF BUNYAVIRIDAE. H.F. LaPenotiere and C.S. Schmaljohn. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 142 CLONING AND SEQUENCE ANALYSIS OF THE M AND S GENOME SEGMENTS OF THE HANTAVIRUS, SR-11 VIRUS. L. Iacono-Connors, H. LaPenotiere, J. Arikawa, and C.S. Schmaljohn. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 143 ANTIGENIC ANALYSIS OF THE STRUCTURAL PROTEINS OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS. J.F. Smith*, N.T. Pesik, L.A. Hodgson, and R. Bethke. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 144 T-LYMPHOCYTE RESPONSES TO HUMAN PAPILLOMA VIRUS PROTEINS. G. Strang* and J. Rothbard. New England Biolabs, Beverly, MA and Imperial Cancer Research Fund, London, UK.

VIRAL DIAGNOSIS

- 145 A DENGUE VIRUS PLAQUE ASSAY UTILIZING THE C6/36 AEDES ALBOPICTUS MOSQUITO CELL LINE. S.E. Hasty*, F.J. Malinoski, W.E. Brandt, and J.M. Dalrymple. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 146 DEVELOPMENT OF A DOT-IMMUNOBINDING ASSAY FOR DETECTING IgM ANTIBODIES TO DENGUE IN ACUTE AND CONVALESCENT SERA. J.J. Oprandy, P.L. Summers, D.J. Gubler, C.G. Hayes, D. Sieckmann, and R. Wistar, Jr. U.S. Naval Medical Research Institute, Bethesda, MD; Walter Reed Army Institute of Research, Washington, DC; Centers for Disease Control, San Juan, PUERTO RICO; and Naval Medical Research Unit No. 2, Manila, THE PHILIPPINES.
- 147 USE OF FLAVIVIRUS - E. COLI FUSION PROTEINS AS DIAGNOSTIC REAGENTS TO DISTINGUISH INFECTIONS BY DIFFERENT FLAVIVIRUSES. W. Fan*, R.E. Shope, B. Fonseca, M.U. Zuegel, M.J. Fournier, T.L. Mason, and P.W. Mason. Yale University School of Medicine, New Haven, CT; and University of Massachusetts, Amherst, MA.

TUESDAY MORNING - DECEMBER 12

POSTER SESSION I (VIRAL DIAGNOSIS - Continued)

- 148 A STUDY OF ANTIBODY RESPONSE TO DENGUE NS1 BY WESTERN BLOT. G. Kuno*, A.V. Vorndam, D.J. Gubler, and I. Gomez. Centers for Disease Control, San Juan, PUERTO RICO.
- 149 OPTIMIZATION AND APPLICATION TO HUMAN DIAGNOSTIC TESTING OF HANTAAN VIRAL ANTIGENS FROM INFECTED CELL CULTURE CELLS. F.R. Bethke*, C.A. Rossi, S. Sebero, J.W. LeDuc, and T.G. Ksiazek. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 150 DIAGNOSTIC POTENTIAL OF A BACULOVIRUS-EXPRESSED, NUCLEOCAPSID PROTEIN FOR HANTAVIRUSES. C.A. Rossi*, C.S. Schmaljohn, and J.W. LeDuc. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 151 RAPID DETECTION OF HEPATITIS A VIRUS IGM ANTIBODIES BY A SOLID PHASE ANTIBODY CAPTURE HEMADSORPTION ASSAY. P.L. Summers*, D.R. Dubois, W.H. Cohen, P.O. Macarthy, L.H. Binn, M.H. Sjogren, R. Snithhan, B.L. Innis, and K.H. Eckels. Walter Reed Army Institute of Research, Washington, DC and Armed Forces Research Institute of Medical Sciences, Bangkok, THAILAND.
- 152 PREPARATION OF NONINFECTIOUS HEPATITIS A VIRUS HEMAGGLUTININ FOR DETECTING HEMAGGLUTINATION INHIBITION ANTIBODIES. D.R. Dubois*, L.N. Binn, P.L. Summers, R.L. Timchak, D.A. Barvir, R.H. Marchwicki, and K.H. Eckels. Walter Reed Army Institute of Research, Washington, DC.

VIRAL PATHOGENESIS

- 153 CRITERIA FOR DETECTION AND QUANTIFICATIONS OF ANTIBODY-DEPENDENT ENHANCEMENT OF INFECTION WITH FLAVIVIRUSES IN VITRO. D.M. Morens and S.B. Halstead. University of Hawaii, Honolulu, HI; Rockefeller Foundation, New York, NY.
- 154 EVALUATION OF MONOCYTE-INFECTIVITY AS AN IN VITRO CORRELATE FOR VIRULENCE OF DENGUE SEROTYPES -1, -3 AND -4. S.C. Kliks*, L.H. Wahl, and D.W. Trent. University of California at Berkeley, Berkeley, CA; National Institutes of Health, Bethesda, MD; and Centers for Disease Control, Fort Collins, CO.
- 155 COMPARATIVE MORPHOGENESIS OF JAPANESE ENCEPHALITIS VIRUS. M.B. Downs*, M.A. Ussery, and T.P. Monath. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

AMEBIASIS AND GIARDIASIS

- 156 FREEZER-PRESERVATION OF NAEGLERIA FOWLERI AMEBAE. D.T. John* and P.L. Eddy. Oral Roberts University School of Medicine, Tulsa, OK.
- 157 A MUTANT OF ENTAMOEB HISTOLYTICA WITH THE COLLAGENASE AND PROTEINASES ACTIVITIES CHANGED. M. De la Garza, J. Serrano, B. Gallegos, R. Tovar, and M.L. Munoz. Centro de Investigacion y de Estudios Avanzados del IPN, MEXICO.

TUESDAY MORNING - DECEMBER 12

POSTER SESSION I (AMEBIASIS AND GIARDIASIS - Continued)

- 158 BINDING OF GROWTH-INHIBITORY TRICYCLIC ANTIDEPRESSANT DRUGS TO TROPHOZOITES OF GIARDIA LAMBLIA. E.C. Weinbach*, L. Levenbook, and D. Rainey. National Institute of Health, Bethesda, MD.
- 159 RECURRENT VARIATIONS OF SURFACE ANTIGENS IN CLONES OF GIARDIA LAMBLIA. J.A. Enciso, R. Fonseca, R. Arguello, R. Cedillo, and M.G. Ortega-Pierres*. Centro de Investigacion y Estudios Avanzados IPN, MEXICO.
- 160 ISOLATION OF CLONES OF GIARDIA LAMBLIA (WB STRAIN) WITH DISTINCT ANTIGENIC AND ISOENZYME PROFILES. I.A. Udezulu*, G.S. Visvesvara, D.M. Moss, and G.J. Leitch. Morehouse School of Medicine, Atlanta, GA and Centers for Disease Control, Atlanta, GA.
- 161 INFECTIVITY TO MONGOLIAN GERBILS AND ISOENZYME PROFILES OF FOUR STRAINS OF GIARDIA LAMBLIA ORIGINATING FROM DIVERSE GEOGRAPHIC AREAS. S. Abaza*, G.S. Visvesvara, and J. Sullivan. Suez Canal University, Ismailia, EGYPT and Centers for Disease Control, Atlanta, GA.
- 162 EVALUATION OF AN ENZYME IMMUNOASSAY (EIA) FOR THE DETECTION OF GIARDIA LAMBLIA IN STOOL SPECIMENS. L. Sloan and J.E. Rosenblatt. The Mayo Clinic and Mayo Foundation, Rochester, MN.

TROPICAL VETERINARY MEDICINE

- 163 USE OF DRIED BLOOD ON FILTER PAPERS AND SERUM SAMPLES FOR SERODIAGNOSIS OF HEMOTROPIC DISEASES--COMPARATIVE STUDY. G.S.Z. Ssenyonga*, S. Montenegro-James, I. Kakoma, and R. Hansen. Makerere University, Kampala, UGANDA; University of Illinois, Urbana, IL.
- 164 STUDIES ON THE MAINTENANCE OF BABESIA BOVIS (MEXICAN ISOLANT) WITHOUT SUBCULTURE USING MICROAEROPHILOUS STATIONARY PHASE (MASP) CULTURE TECHNIQUE. A.K. Mishra*, G. Clabaugh, I. Kakoma, and M. Ristic. University of Illinois, Urbana, IL.
- 165 THE ANTIGENICITY AND IMMUNOGENICITY OF THEILERIA SERGENTI ISOLATED FROM KOREAN CATTLE. B.K. Baek*, B.S. Kim, J.H. Kim, C.M. Chin, S. Montenegro-James, and I. Kakoma. Chonbuk National University, Chonju, KOREA; University of Illinois, Urbana, IL.
- 166 THE CLONING AND EXPRESSION OF THE CAPSULAR ANTIGEN (F1) OF YERSINIA PESTIS IN ESCHERICHIA COLI AND ITS POTENTIAL USE IN SERODIAGNOSIS AND VACCINE DEVELOPMENT. W.J. Simpson*, R.E. Thomas, T.G. Schwan, and M.E. Schrupf. National Institutes of Health, Hamilton, MT.
- 167 INTRAGASTRIC INOCULATION OF THE PLAGUE CAPSULAR ANTIGEN CAN RESULT IN NONPROTECTIVE SERUM TITERS IN MICE. R.E. Thomas* and T.G. Schwan. National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT.

TUESDAY MORNING - DECEMBER 12

SYMPOSIUM: CYTOADHERENCE AND CEREBRAL MALARIA*

10:00 AM - 12:00 Noon

Coral III

Chairpersons: L.H. Miller and I.J. Udeinya

Time Abstract

10:00	168	INTRODUCTION: SEQUESTRATION OF MALARIA-INFECTED ERYTHROCYTES IN VIVO. L.H. Miller. National Institutes of Health, Bethesda, MD.
10:05	169	PATHOLOGY OF HUMAN CEREBRAL MALARIA. M. Aikawa. Case Western Reserve University. Cleveland, OH.
10:25	170	IN VITRO AND EX-VIVO SEQUESTRATION ASSAYS. I.J. Udeinya. Walter Reed Army Institute of Research, Washington, DC.
10:45	171	INFECTED ERYTHROCYTE SURFACE MOLECULES INVOLVED IN CYTOADHERENCE. R.H. Howard. DNAX Research Institute. Palo Alto, CA.
11:05	172	HOST RECEPTORS FOR INFECTED ERYTHROCYTES. J. Chulay. Walter Reed Army Institute of Research, Washington, DC.
11:25	173	CEREBRAL MALARIA IN CHILDREN: CLINICAL IMPLICATIONS OF CYTOADHERENCE. M. Molyneux. Malaria Research Project, Blantyre, MALAWI.
11:50		DISCUSSION AND CLOSING COMMENTS.

* - This symposium on Cytoadherence and Cerebral Malaria is supported by the Agency for International Development Malaria Immunity and Vaccine Research Program.

SCIENTIFIC SESSION D: CLINICAL TROPICAL MEDICINE

10:00 AM - 12:00 Noon

Coral IV

Chairpersons: M.S. Wolfe and R.S. Goldsmith

Time Abstract

10:00	174	SKELETAL AND CARDIAC MUSCLE INVOLVEMENT IN SEVERE, LATE LEPTOSPIROSIS. G. Watt*, L.P. Padre and M.L. Tuazon. Naval Medical Research Unit No. 2, Manila, THE PHILIPPINES.
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TUESDAY MORNING - DECEMBER 12

SCIENTIFIC SESSION D: CLINICAL TROPICAL MEDICINE (Continued)

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| 10:15 | 175 | LATE STAGE EAST AFRICAN HUMAN TRYPANOSOMIASIS: TREATMENT AND FOLLOW-UP. J.D. Bales*, S.M. Harrison, D.L. Mbwabi, K.M. Tengekyon, A.R. Njogu, and P.J. Schechter. Fitzsimmons Army Medical Center, Aurora, CO; Kenya Trypanosomiasis Research Institute, Nairobi, KENYA; Merrell Dow Research Institute, Strasbourg, FRANCE. |
| 10:30 | 176 | KATAYAMA FEVER IN U.S. TRAVELERS RETURNING FROM BOTSWANA: POSSIBLE ROLE OF A HYBRID SCHISTOSOME SPECIES. M.K. Michelson*, J.J. Sullivan, L. Chitsulo, M. Wilson, H.S. Bishop, D.D. Juraneck, and H.C. Spencer. Centers for Disease Control, Atlanta, GA. |
| 10:45 | 177 | MALARIA IN ADULTS AT A MAJOR MEDICAL CENTER IN NEW YORK CITY, 1971-1987. J.M. Courval* and S.H. Vermund. Columbia University, New York, NY; National Institute of Allergy and Infectious Disease, Bethesda, MD. |
| 11:00 | 178 | INTRAMUSCULAR QUININE IN THE TREATMENT OF SEVERE PEDIATRIC <u>FALCIPARUM</u> MALARIA. T.E. Taylor*, M.E. Molyneux, and J.J. Wirima. Michigan State University; Liverpool School of Tropical Medicine, UK; and Kamuzu Central Hospital, Lilongwe, MALAWI. |
| 11:15 | 179 | GLUCOSE METABOLISM IN QUININE-TREATED <u>FALCIPARUM</u> MALARIA. T.M.E. Davis*, W. Supanaranond, S. Pukrittayakamee, S. Krishna, S. Looareesuwan, R.C. Turner, and N.J. White. Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, THAILAND; Nuffield Department of Clinical Medicine and Diabetes Research Laboratories; Oxford University, Oxford, UK. |
| 11:30 | 180 | MALARIA CHEMOPROPHYLAXIS WITH PROGUANIL/SULFAMETHOXAZOLE. J.J. Karwacki, G.D. Shanks*, V. Suriyamongkok, and C. Watanasook. Armed Forces Research Institute of Medical Sciences, Bangkok, THAILAND and Royal Thai Navy Medical Department, THAILAND. |
| 11:45 | 181 | PERMETHRIN-IMPREGNATED CURTAINS AND BED NETS PREVENT MALARIA IN WESTERN KENYA. J.D. Sexton*, T.K. Ruebush II, A.D. Brandling-Bennett, J.G. Breman, S.J. Odera, J.M. Roberts and J.B.O. Were. Centers for Disease Control, Atlanta, GA; and Kenya Medical Research Institute, Nairobi, KENYA. |

TUESDAY MORNING - DECEMBER 12

SCIENTIFIC SESSION E: ARBOVIRAL ENTOMOLOGY

10:00 AM - 12:00 Noon

South Pacific I - II

Chairpersons: B. Beaty and M. Wilson

Time Abstract

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| 10:00 | 182 | VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION IN AND TRANSMISSION BY <u>AMBLIOMMA CAJENNEENSE</u> (FABRICIUS). K.J. Linthicum*, T.M. Logan, C.L. Bailey, S.W. Gordon, C.J. Peters, T.P. Monath, J. Osario, D.B. Francly, R.G. McLean, J. LeDuc, R.R. Graham, J. Moulton, and D. Dohm. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MA and the Centers for Disease Control, Fort Collins, CO. |
| 10:15 | 183 | DETECTION OF ARBOVIRUS DEPOSITION IN MOSQUITOES FOLLOWING INGESTION OF RADIOLABLED VIRUS IN BLOOD MEALS. S.C. Weaver,* T.W. Scott, L.H. Lorenz, and P.M. Repik. University of Maryland, College Park, MD; and Medical College of Pennsylvania, Philadelphia, PA. |
| 10:30 | 184 | NUMBER OF EGGS PER SITE LAID BY <u>AEDES AEGYPTI</u> AND IMPLICATIONS FOR DISPERSAL AND VIRUS TRANSMISSION. P. Reiter*, M.A. Amador and D.J. Gubler. Centers for Disease Control, San Juan, PUERTO RICO. |
| 10:45 | 185 | TRANSFILIAL TRANSMISSION STUDIES OF ST. LOUIS ENCEPHALITIS VIRUS IN <u>AEDES TAENIORYNCHUS</u> AND <u>PSOROPHORA TOLTECUM</u> . B.S. Des Rochers* and J.L. Hardy. University of California at Berkeley, Berkeley, CA. |
| 11:00 | 186 | TRANSMISSION OF RIFT VALLEY FEVER VIRUS BY THE SAND FLY <u>PHLEBOTOMUS DUBOSQI</u> . M.J. Turell and P.V. Perkins. U.S. Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; and Walter Reed Army Institute of Research, Washington, DC. |
| 11:15 | 187 | CRIMEAN-CONGO HEMORRHAGIC FEVER IN SENEGAL: TEMPORAL AND SPATIAL PATTERNS OF INFECTION RELATED TO VECTOR BIOLOGY. M.L. Wilson*, B. LeGuerno, J.P. Gonzalez, J.P. Cornet, and J.L. Camicas. Institut Pasteur, Dakar, SENEGAL; U.S. Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; Laboratoire ORSTOM de Zoologie Medicale, Institut Pasteur, Dakar, SENEGAL. |
| 11:30 | 188 | REPLICATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS (FAMILY BUNYAVIRIDAE, GENUS NAIROVIRUS) IN FOUR SPECIES OF IXODID TICKS (ACARI: IXODIDAE) AFTER EXPERIMENTAL INFECTION. T.M. Logan*, K.J. Linthicum, C.L. Bailey, D.M. Watts, D.J. Dohm, and J.R. Moulton. U.S. Army Medical Research Unit-KENYA; U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; U.S. Navy Medical Research Unit, No. 3, Cairo, EGYPT. |

TUESDAY MORNING - DECEMBER 12

SCIENTIFIC SESSION E: ARBOVIRAL ENTOMOLOGY (Continued)

- 11:45 189 ISOFEMALE LINE ANALYSES OF THE GENETIC BASIS OF ORAL SUSCEPTIBILITY OF CULICOIDES VARIIPENNIS FOR BLUETONGUE VIRUS. W.J. Tabachnick* and J.O. Mecham. U.S. Department of Agriculture, Agricultural Research Service, Arthropod-Borne Animal Diseases Research Laboratory, Laramie, WY.

SCIENTIFIC SESSION F: SCHISTOSOMIASIS - IMMUNOPATHOGENESIS AND MECHANISMS OF IMMUNITY

10:00 AM - 12:00 Noon

South Pacific III - IV

Chairpersons: A. Sher and F. Von Lichtenberg

Time Abstract

- 10:00 190 CHARACTERIZATION OF TH1 VERSUS TH2 CELL FUNCTION IN MICE VACCINATED WITH RADIATION-ATTENUATED CERCARIAE. S.L. James, F. Al-Zamel, Z. Caulada, and E. Pearce. Biomedical Research Institute, Rockville, MD; George Washington University Medical Center, Washington, DC; National Institutes of Health, Bethesda, MD.
- 10:15 191 SELECTIVE PRODUCTION OF CYTOKINES ASSOCIATED WITH THE RESPONSE BY SPLENOCYTES, DRAINING LYMPH NODE CELLS AND ISOLATED GRANULOMAS DURING ACUTE MURINE INFECTION WITH SCHISTOSOMA MANSONI. J.M. Grzych*, E.J. Pearce, A.W. Cheever, P. Scott, and A. Sher. National Institutes of Health, Bethesda, MD.
- 10:30 192 IL-2 PRODUCTION, RESPONSIVENESS, UTILIZATION AND RECEPTOR DISPLAY BY SPLEEN CELLS OF S. MANSONI INFECTED MICE. T. Yamashita and D.L. Boros*. Wayne State University School of Medicine, Detroit, MI.
- 10:45 193 CHARACTERIZATION OF A HUMAN SERUM FACTOR THAT INHIBITS CYTOKINE MEDIATED ACTIVATION OF HUMAN EOSINOPHILS AND NEUTROPHILS. M.S. Minkoff*, S. McDonough and D.S. Silverstein. Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA.
- 11:00 194 HUMAN EOSINOPHIL CYTOTOXICITY-ENHANCING FACTOR (ECEP): PURIFICATION, N-TERMINAL AMINO ACID SEQUENCE, IDENTIFICATION OF SECRETED AND CELL-ASSOCIATED FORMS. D.S. Silverstein* and J.R. David. Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA.
- 11:15 195 SCHISTOSOMA MANSONI: EFFECTS OF PRAZIQUANTEL ON CAPPING AND CALCIUM UPTAKE IN ADULT WORMS. R.E. Akridge*, S. Anglin, T.A. Badar, and W.M. Kemp. Texas A & M University, College Station, TX.

TUESDAY MORNING - DECEMBER 12

SCIENTIFIC SESSION F: SCHISTOSOMIASIS - IMMUNOPATHOGENESIS
AND MECHANISMS OF IMMUNITY (Continued)

- 11:30 196 PURIFICATION OF SCHISTOSOMAL EGG-GRANULOMA DERIVED FIBROBLAST GROWTH FACTOR. S. Prakash* and D.J. Wyler. New England Medical Center Hospitals and Tufts University School of Medicine, Boston, MA.
- 11:45 197 INDUCTION OF T HELPER CELL HYPORESPONSIVENESS TO ANTIGEN BY MACROPHAGES FROM SCHISTOSOMAL EGG GRANULOMAS: A BASIS FOR IMMUNOMODULATION IN SCHISTOSOMIASIS. J. K. Kamisato and M.J. Stadecker. Tufts University School of Medicine, Boston, MA.

SCIENTIFIC SESSION G: KINETOPLASTIDIA - IMMUNOLOGY

10:00 AM - 12:00 Noon

Nautilus II - III

Chairperson: S.G. Reed

Time Abstract

- 10:00 198 A VIABLE POPULATION OF L3T4+ (CD4+) T-LYMPHOCYTES IS NECESSARY FOR DEVELOPMENT OF RESISTANCE TO TRYPANOSOMA CRUZI. F.G. Araujo*. Research Institute, Palo Alto Medical Foundation, Palo Alto, CA.
- 10:15 199 THE GP63 GENE OF LEISHMANIA DONOVANI CHAGASI LACKS THE RGD MACROPHAGE BINDING LIGAND. R.A. Miller*, M. Parsons, and S.G. Reed. Seattle Biomedical Research Institute, Seattle, WA.
- 10:30 200 THE ROLE OF TUMOR NECROSIS FACTOR IN EXPERIMENTAL MURINE CUTANEOUS LEISHMANIASIS. C.M. Theodos*, R.M. Molina and R.G. Titus. Harvard School of Public Health, Boston, MA.
- 10:45 201 PATTERN OF LYMPHOKINE SECRETION IN MURINE LEISHMANIASIS: CORRELATION WITH DISEASE PROGRESSION. W.H. Boom, L. Liebster, A.K. Abbas and R.B. Titus*. Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA.
- 11:00 202 EVIDENCE FOR TWO DISTINCT PATHWAYS OF MACROPHAGE ACTIVATION FOR ANTILEISHMANIAL DEFENSE. J.P. Sypek* and D.J. Wyler. New England Medical Center Hospitals and Tufts University School of Medicine, Boston, MA.
- 11:15 203 LEISHMANIA MAJOR PROVIDES THE TRIGGER SIGNAL FOR MICROBICIDAL AND TUMORICIDAL EFFECTOR ACTIVITIES OF INTERFERON PRIMED MACROPHAGES. R.M. Crawford*, S.J. Green, M.S. Meltzer, and C.A. Nacy. Walter Reed Army Institute of Research, Washington, DC.

TUESDAY MORNING - DECEMBER 12

SCIENTIFIC SESSION G: KINETOPLASTIDIA - IMMUNOLOGY (Continued)

- 11:30 204 HUMAN T CELLS RECOGNIZE LEISHMANIAL ANTIGENS ON INFECTED MONOCYTES. P.C. Melby, G. Collet-Lima, and D.L. Sack*. National Institutes of Health, Bethesda, MD.
- 11:45 205 HUMAN T-LYMPHOCYTE RESPONSES TO GP63, A MAJOR SURFACE ANTIGEN OF LEISHMANIA. D.M. Russo*, E.M. Carvalho, D.M. Mosser, R. McMaster, and S.G. Reed. Seattle Biomedical Research Institute, Seattle, WA; Federal University of Bahia, Salvador, BRAZIL; Temple University School of Medicine, Philadelphia, PA; University of British Columbia, Vancouver, CANADA.

TUESDAY AFTERNOON - DECEMBER 12

SCIENTIFIC SESSION H: VIRAL PATHOGENESIS

1:30 PM - 3:00 PM

South Pacific I - II

Chairpersons: K.M. Johnson and B.L. Innis

Time Abstract

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| 1:30 | 206 | IMMUNITY TO DEN (DEN) PROTECTS AGAINST DEATH DUE TO JAPANESE ENCEPHALITIS (JE). B.L. Innis, A. Nisalak, C.H. Hoke, S. Suntayakorn, P. Puttisri, V. Chongswasdi, C. Kaemkosolsri, N. Mimpitakpongs and P. Kiatbumpen. Armed Forces Research Institute of Medical Sciences, Bangkok, THAILAND; and Ministry of Public Health, THAILAND. |
| 1:45 | 207 | BIOLOGICAL MECHANISM RELATED TO VIRULENCE OF DENGUE-2 VIRUS. S.C. Kliks*, R.S. Alfonso, and J.L. Hardy. School of Public Health, University of California, Berkeley, CA. |
| 2:00 | 208 | BUNYAVIRUS-VECTOR INFECTION MODEL: MONOCLONAL ANTIBODIES DIRECTED AGAINST THE G2 GLYCOPROTEIN OF A LA CROSSE VIRUS. G.V. Ludwig*, T.M. Yuill, B.M. Christensen, and K.T. Schultz. University of Wisconsin, Madison, WI. |
| 2:15 | 209 | DUGBE VIRUS SUSCEPTIBILITY TO NEUTRALISATION BY MONOCLONAL ANTIBODIES AS A MARKER OF VIRULENCE IN MICE. A. Buckley and E.A. Gould. NERC Institute of Virology, Oxford, UK. |
| 2:30 | 210 | COMPARISON OF THOGOTO VIRUS INFECTION IN A PERMISSIVE AND APPARENTLY NON-PERMISSIVE VERTEBRATE. L.D. Jones*, T. Booth, and P.A. Nuttall. NERC, Institute of Virology and Environmental Microbiology, Oxford, UK. |
| 2:45 | 211 | TRANSMISSION OF HEPATITIS E VIRUS TO OWL MONKEYS (AOTUS TRIVIRGATUS). J. Ticehurst*, L. Rhodes, K. Krawczynski, L. Asher, W. Engler, J. Caudill, M. Sjogren, C. Hoke, J.W. LeDuc, D. Bradley, and L. Binn. Walter Reed Army Institute of Research, Washington, DC; U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick MD; Centers for Disease Control, Atlanta, GA; Armed Forces Institute of Pathology, Washington, DC. |

SCIENTIFIC SESSION I: MALARIA - MOLECULAR BIOLOGY

1:15 PM - 3:15 PM

South Pacific III - IV

Chairpersons: M. Gross and D. Harrington

Time Abstract

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| 1:15 | 212 | A GENE THAT ENCODES FOR AN ERYTHROCYTE BINDING PROTEIN OF <u>PLASMODIUM KNOWLESI</u> MEROZOITES. J.H. Adams, D.E. Hudson, T.E. Wellems, and L.H. Miller. National Institutes of Health, Bethesda, MD. |
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TUESDAY AFTERNOON - DECEMBER 12

SCIENTIFIC SESSION I: MALARIA - MOLECULAR BIOLOGY (Continued)

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| 1:30 | 213 | HEAT SHOCK PROTEINS OF <u>PLASMODIUM BERGHEI</u> AND <u>P. FALCIPARUM</u> SPOROZOITES AND EXOERYTHROCYTIC PARASITES. N. Kumar*, M. Aikawa, C. Atkinson, J.F.G.M. Meis, B. Sina, A. Appiah, and M.R. Hollingdale. Johns Hopkins University, Baltimore, MD; Case Western Reserve University, Cleveland, OH; University Nijmegen, THE NETHERLANDS; and Biomedical Research Institute, Rockville, MD. |
| 1:45 | 214 | USE OF THE POLYMERASE CHAIN REACTION (PCR) TO AMPLIFY MALARIA DNA AS A TARGET FOR DNA PROBES. R.H. Barker, Jr.* and D.F. Wirth. Harvard School of Public Health, Boston, MA. |
| 2:00 | 215 | MAPPING B EPITOPES OF THE <u>PLASMODIUM FALCIPARUM</u> PFS 25 OOKINETE PROTEIN AND THE PRODUCTION OF SYNTHETIC PEPTIDE ANTIBODIES REACTIVE TO THE NATIVE PROTEIN: IMPLICATIONS FOR VACCINE DEVELOPMENT. I.A. Quakyi, D.C. Kaslow, M.F. Good, S.N. Isaacs, J.H. Nunberg, J.A. Berzofsky, B. Moss, R. Houghton, D.B. Keister, and L.H. Miller. National Institutes of Health, Bethesda, MD; Cetus Corporation, Emeryville, CA; Scripps Clinic and Research Foundation, La Jolla, CA. |
| 2:15 | 216 | USE OF SYNTHETIC DNA OLIGOMERS TO ANALYZE THE REPETITIVE DNA FAMILIES OF <u>PLASMODIUM FALCIPARUM</u> . G.L. Laughlin*. University of Illinois, Urbana, IL. |
| 2:30 | 217 | CLONING, SEQUENCING AND EXPRESSION OF A 17 AMINO ACID REPEAT ANTIGEN GENE FROM <u>PLASMODIUM FALCIPARUM</u> EE PARASITES. J. Zhu, B. Sina, K. Sakhuja, A. Lal, and M.R. Hollingdale. Biomedical Research Institute, Rockville, MD. |
| 2:45 | 218 | IMMUNOGENICITY OF <u>PLASMODIUM FALCIPARUM</u> AND VIVAX CIRCUMSPOROZOITE PROTEINS, AND THEIR ANALOGS, PRODUCED IN YEAST. I.C. Bathurst*, B. Hahm, J. Kansopon, H.L. Gibson, K.M. Green, W.E. Collins, M.R. Hollingdale, E.H. Nardin, R.S. Nussenzweig, V. Nussenzweig, and P.J. Barr. Chiron Corporation, Emeryville, CA; Centers for Disease Control, Atlanta, GA; Biomedical Research Institute, Rockville, MD; and New York University Medical Center, New York, NY. |
| 3:00 | 219 | EXPRESSION IN YEAST OF PFS25, A SEXUAL STAGE ANTIGEN OF <u>PLASMODIUM FALCIPARUM</u> . H.L. Gibson, K.M. Green, J.W. Tung, K.S. Steimer, D.C. Kaslow, and P.J. Barr*. Chiron Corporation, Emeryville, CA and National Institutes of Health, Bethesda, MD. |

TUESDAY AFTERNOON - DECEMBER 12

SCIENTIFIC SESSION J: GIARDIASIS AND TOXOPLASMOSIS

1:30 PM - 3:00 PM

Nautilus II - III

Chairpersons: J.K. Frenkel and T.E. Nash

<u>Time</u>	<u>Abstract</u>	
1:30	220	EXCYSTATION OF IN VITRO DERIVED <u>GIARDIA LAMBLIA</u> CYSTS. S.E.M. Boucher, D.S. Reiner, S. Das, and F.D. Gillin*. University of California at San Diego, San Diego, CA.
1:45	221	RESTRICTED VARIANT SURFACE EPITOPES ON <u>GIARDIA</u> . T.E. Nash, J.T. Conrad and J.W. Merritt, Jr. National Institutes of Health, Bethesda, MD.
2:00	222	IDENTIFICATION OF MULTIPLE <u>GIARDIA LAMBLIA</u> ANTIGENS FROM HUMAN STOOL. H.M. Mathews*, D.M. Moss, and G.S. Visvesvara. Centers for Disease Control, Atlanta, GA.
2:15	223	SEROTYPING OF GEOGRAPHICALLY DISTINCT HUMAN <u>GIARDIA LAMBLIA</u> . E.W. Mohareb*, J.I. Bruce, and J.B. Hughes. University of Lowell Center for Tropical Disease, Lowell, MA.
2:30	224	CHARACTERIZATION AND cDNA CLONING OF A 22 KD SURFACE ANTIGEN (P22) OF <u>TOXOPLASMA GONDII</u> (Tg). J.B. Prince*, J. Huskinson, K.L. Auer, and J.S. Remington. Research Institute, Palo Alto Medical Foundation, Palo Alto, CA and Stanford University School of Medicine, Stanford, CA.
2:45	225	A REPRODUCTIVELY-DEFICIENT MUTANT <u>TOXOPLASMA</u> VACCINE FOR CATS. J.K. Frenkel*, E.R. Pfefferkorn, J.L. Fishback, and D.D. Smith. University of Kansas School of Medicine, Kansas City, KS and Dartmouth Medical School, Hanover, NH.

SCIENTIFIC SESSION K: TROPICAL VETERINARY MEDICINE

1:30 PM - 3:00 PM

Sea Pearl II - III

Chairperson: J. C. Williams

<u>Time</u>	<u>Abstract</u>	
1:30	226	T-LYMPHOCYTE PROLIFERATIVE RESPONSES AND CORRELATION OF CROSS-PROTECTION AGAINST VIRULENT <u>RICKETTSIA RICKETTSII</u> BY IMMUNIZATION WITH <u>RICKETTSIA RHIPICEPHALI</u> . K.L. Gage, T.R. Jerrells, and D.H. Walker.* University of Texas Medical Branch, Galveston, TX.
1:45	227	ANTIGENIC ANALYSIS OF <u>EHRlichia CANIS</u> AT THE POLYPEPTIDE LEVEL: IDENTIFICATION OF SPECIES-SPECIFIC ANTIGENS FOR USE IN THE DIAGNOSIS OF CANINE EHRlichiosis. S.J. Ma, C.J. Holland*, I. Kakoma, N. Rajapakse, and M. Ristic. University of Illinois, Urbana, IL.

TUESDAY AFTERNOON - DECEMBER 12

SCIENTIFIC SESSION K: TROPICAL VETERINARY MEDICINE (Continued)

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| 2:00 | 228 | INTRACELLULAR MICROORGANISMS IN <u>DIROFILARIA IMMITIS</u> . W. J. Kozek*. University of Puerto Rico, San Juan, PUERTO RICO. |
| 2:15 | 229 | DOT-ELISA FOR SERODIAGNOSIS OF ANAPLASMOSIS AND BABESIOSIS. S. Montenegro-James*, A.T. Guillen, S.J. Ma, P. Tapang, and M. Ristic. University of Illinois, Urbana, IL; Instituto de Investigaciones Veterinarias, FONAIAP, Maracay, VENEZUELA. |
| 2:30 | 230 | SURVEILLANCE OF HUMAN EHRLICHIOSIS IN THE UNITED STATES, 1988. T.R. Eng*, D.B. Fishbein, J.E. Dawson, N. Greene, and M. Redus. Centers for Disease Control, Atlanta, GA. |
| 2:45 | 231 | IMMUNOGENICITY OF A SYNTHETIC PEPTIDE DERIVED FROM THE BabR LOCUS OF <u>BABESIA BOVIS</u> . G.L. McLaughlin*, M.A. James, M. Toro, and S. Montenegro-James. University of Illinois, Urbana, IL and Instituto de Investigaciones Veterinarias Agropecuarias, VENEZUELA. |

SCIENTIFIC SESSION L: ENTOMOLOGY - SANDFLIES

1:30 PM - 3:00 PM

Sea Pearl IV - VI

Chairperson: R.B. Tesh

- | <u>Time</u> | <u>Abstract</u> | |
|-------------|-----------------|--|
| 1:30 | 232 | CHINKS IN THE BEHAVIORAL ARMATURE OF <u>PHLEBOTOMUS PAPATASI</u> (DIPTERA: PSYCHODIDAE), THE VECTOR OF LEISHMANIASIS IN ISRAEL. B. Yuval, A. Warburg and Y. Schlein. Hebrew University Medical School, Jerusalem, ISRAEL. |
| 1:45 | 233 | ON THE RECOGNITION MECHANISM BETWEEN LEISHMANIA FLAGELLA AND SAND-FLY MIDGUT EPITHELIUM. A. Warburg*, R.B. Tesh and D. McMahon-Pratt. Yale School of Medicine, New Haven, CT. |
| 2:00 | 234 | RECOVERY OF <u>BARTONELLA BACILLIFORMIS</u> FROM CRYOPRESERVED <u>LUTZOMYIA VERRUCARUM</u> AND <u>L. PERUENSIS</u> (DIPTERA: PSYCHODIDAE). C.R. Latorre*, E. Rogers, S.E. Romero, and R. Fernandez. U.S. Medical Research Institute Detachment, Lima, PERU. |
| 2:15 | 235 | SAND FLIES ASSOCIATED WITH HUMAN POPULATIONS IN THE REPUBLIC OF DJIBOUTI. D.J. Fryauff, H.A. Hanafi, and C. Bailly. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT; Service d'Hygiene, Ministre de Sante et Affaires Sociales, REPUBLIQUE DE DJIBOUTI. |
| 2:30 | 236 | BIOCHEMICAL TAXONOMY, ENZYME POLYMORPHISM, AND POPULATION GENETICS OF EGYPTIAN SAND FLY VECTORS OF LEISHMANIA. H.A. Kassem,* D.J. Fryauff, M.G. Shehata, and B.M. El Sawaf. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT; Training Center for Research on Vectors of Diseases, Ain Shams University, Cairo, EGYPT. |

SCIENTIFIC SESSION L: ENTOMOLOGY - SANDFLIES (Continued)

ASTMH PRESIDENTIAL ADDRESS

Coral III - IV

3:35 238 BIOLOGY OF MALARIA. L.H. Miller. Malaria Section,
Laboratory of Parasitic Diseases, National Institutes of
Allergy and Infectious Diseases, National Institutes of
Health, Bethesda, MD.

ASTMH ANNUAL BUSINESS MEETING

Coral III - IV

Chairpersons: W.A. Sodeman and L.H. Miller

WEDNESDAY MORNING - DECEMBER 13

POSTER SESSION II WITH CONTINENTAL BREAKFAST

7:30 AM - 10:30 AM

Coral Lounge

AUTHORS IN ATTENDANCE FROM 8:00 AM - 10:00 AM

The poster boards will be available in the Coral Lounge beginning at 7:00 PM Tuesday evening. Poster should be set up by 8:00 AM Wednesday morning and taken down by 5:00 PM Wednesday afternoon.

MALARIA - BLOOD STAGES

- 239 RELEASE OF MEROZOITE DENSE GRANULES DURING ERYTHROCYTE INVASION BY PLASMODIUM KNOWLESI. M. Torii*, J.H. Adams, L.H. Miller, and M. Aikawa. Case Western Reserve University and National Institutes of Health, Bethesda, MD.
- 240 OCTAPEPTIDE EPITOPES IN PLASMODIUM FALCIPARUM CAMP STRAIN MEROZOITE SURFACE ANTIGEN DETECTED WITH AOTUS ANTIBODIES. J.M. Carter*, A.W. Thomas, and J.A. Lyon. Walter Reed Army Institute of Research, Washington, DC.
- 241 COMPARISON OF INVASION EFFICIENCIES OF ERYTHROCYTES FROM DIFFERENT MOUSE STRAINS BY PLASMODIUM FALCIPARUM MEROZOITES. F.W. Klotz*, P. Orlandi, J.D. Haynes, S.J. Cohen, G. Reuter, R. Schauer, R.J. Howard, and L.H. Miller. Walter Reed Army Institute of Research, Washington, DC; Biochemisches Institut, Christina-Albrecht's Universitat, Kiel, WEST GERMANY; DNAX, Palo Alto, CA; and the National Institutes of Health, Bethesda, MD.
- 242 ERYTHROPOIESIS DURING LETHAL AND NONLETHAL PLASMODIUM YOELII INFECTIONS IN MICE. K.L. Miller*, P.H. Silverman and B. Kullgren. Lawrence Berkeley Laboratory, University of California, Berkeley, CA.
- 243 DEFINED PLASMA-FREE MEDIA FOR THE CULTIVATION OF PLASMODIUM FALCIPARUM. V.C. Okoye and S.K. Martin*. Walter Reed Army Institute of Research, Washington, DC.
- 244 A NON-SIALIC ACID DEPENDENT PATHWAY OF INVASION CAN BE INDUCED IN A PLASMODIUM FALCIPARUM CLONE BY CULTIVATION IN NEURAMINADASE-TREATED ERYTHROCYTES. S.A. Dolan*, L.H. Miller, and T.E. Wellems. National Institutes of Health, Bethesda, MD.
- 245 HUMAN IMMUNE RESPONSE TO MALARIAL HEAT SHOCK PROTEINS. Y. Zhao*, P. Graves, and N. Kumar. Johns Hopkins University, Baltimore, MD and Q.I.M.R., Brisbane, AUSTRALIA.
- 246 CHARACTERIZATION OF ANTIBODY RESPONSES INDUCED BY DIFFERENT SYNTHETIC ADJUVANTS TO THE P. FALCIPARUM MAJOR MEROZOITE SURFACE PRECURSOR PROTEIN, GP195. G.S.N. Hui, S.P. Chang, L.Q. Tam, A. Kato, S.E. Case*, C. Hashiro, S. Kotani, T. Shiba, S. Kusumoto, and W.A. Siddiqui. University of Hawaii, Honolulu, HI; Osaka College of Medical Technology, Osaka, JAPAN.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (MALARIA - BLOOD STAGES - Continued)

- 247 GROWTH INHIBITORY ANTIBODIES INDUCED IN RABBITS USING THE COMBINATION OF SYNTHETIC ADJUVANTS B30-MDP AND LA-15-PH WITH GP195, A PROMINENT MALARIA VACCINE CANDIDATE. L.Q. Tam*, G.S.N. Hui, J. Kagemoto, S. Kotani, T. Shiba, S. Kusumoto, and W.A. Siddiqui. University of Hawaii, Honolulu, HI and Osaka College of Medical Technology, Osaka, JAPAN.
- 248 A NOVEL 230 KD PLASMODIUM FALCIPARUM ANTIGEN MAY BE A TARGET OF IMMUNE ATTACK. I.N. Ploton*, A.W. Thomas, J.M. Carter, and J.A. Lyon. Walter Reed Army Institute of Research, Washington, DC; Smith Kline Beckman, King of Prussia, PA.
- 249 IDENTIFICATION AND CHARACTERIZATION OF THE 50 KD ANTIGEN INVOLVED IN PLASMODIUM FALCIPARUM ICM FORMATION. D.A. Carr*, J.A. Lyon, and A.W. Thomas. Walter Reed Army Institute of Research, Washington, DC.
- 250 SEQUENCE DIVERGENCE BETWEEN THE ERYTHROCYTE BINDING ANTIGENS (EBA-175) OF THE CAMP AND FCR-3 STRAINS OF PLASMODIUM FALCIPARUM. L.A. Medvitz* and D.E. Lanar. Walter Reed Army Institute of Research, Washington, DC.
- 251 INDIVIDUALS PRODUCE DIFFERENT REPERTOIRES OF ANTIBODIES THAT INHIBIT MALARIA MEROZOITE DISPERSAL. J.A. Lyon* and A.W. Thomas. Walter Reed Army Institute of Research, Washington, DC.
- 252 IDENTIFICATION OF A NOVEL 4 KB REPETITIVE UNIT FROM PLASMODIUM FALCIPARUM. J.R. Plitt, G.X. Chen, A.K. Weiler, J. Zhu, and J.W. Zolg*. Biomedical Research Institute, Rockville, MD and University of Dusseldorf, Dusseldorf, WEST GERMANY.
- 253 BLOOD STAGE PLASMODIUM YOELII INFECTION DOES NOT STIMULATE PROTECTIVE CD8+ T CELL IMMUNE RESPONSES. J.M. Vinetz, S. Kumar, M.F. Good, J.A. Berzofsky, and L.H. Miller. National Institutes of Health, Bethesda, MD.
- 254 EXPRESSION OF DOMAINS OF PLASMODIUM FALCIPARUM GP195 IN YEAST. H.L. Gibson*, I.C. Bathurst, P.J. Barr, G.S.N. Hui, S.P. Chang, and W.A. Siddiqui. Chiron Corporation, Emeryville, CA and University of Hawaii, Honolulu, HI.
- 255 IDENTIFICATION OF PLASMODIUM CHABAUDI ADAMI ANTIGENS WHICH ELICIT PROTECTIVE IMMUNITY IN MICE. P.E. Kima* and C.A. Long. Hahnemann University, Philadelphia, PA.
- 256 RECOMBINANT SERA ANTIGENS PRODUCED IN THE YEAST SACCHAROMYCES CEREVISIAE. K.M. Green*, H.L. Gibson, I.C. Bathurst, P.J. Barr, W. Li, D.J. Bzik, and J. Inselberg. Chiron Corporation, Emeryville, CA and Dartmouth Medical School, Hanover, NH.
- 257 IMMUNOGENICITY AND PROTECTIVE EFFICACY OF PLASMODIUM FALCIPARUM CULTURE-DERIVED EXOANTIGENS. I. Kakoma*, M.A. James, C. Fajfar-Whetstone, P. Buese, R. Hansen, G. Clabaugh, and M. Ristic. College of Veterinary Medicine, University of Illinois, Urbana, IL.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (MALARIA - BLOOD STAGES - Continued)

- 258 USE OF SYNTHETIC PEPTIDES IN THE DETECTION OF PLASMODIUM FALCIPARUM BLOOD-STATE ANTIBODIES IN RESIDENTS OF BOLIVAR STATE, VENEZUELA. M.A. James*, S. Montenegro-James, O. Noya, and F. Riggione. Tulane University Medical Center, New Orleans, LA and Universidad Central de Venezuela, Instituto de Medicina Tropical, Caracas, VENEZUELA.
- 259 EPIDEMIOLOGY AND IMMUNOLOGY OF P. FALCIPARUM MALARIA IN A POPULATION CONTAINING IMMUNE AND NONIMMUNE ADULT SUBJECTS. T.R. Jones*, J.K. Baird, S. Ratiwayanto, H. Hadiputranto, B. Leksana, and H. Basri. U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta, INDONESIA.
- 260 AGE-DEPENDENT, CHRONIC EXPOSURE-INDEPENDENT HUMORAL IMMUNE RESPONSES TO HYPERENDEMIC FALCIPARUM MALARIA IN ARSO PIR, IRIAN JAYA. J.K. Baird*, T.R. Jones, M.J. Bangs, S. Tirtokusumo, and I. Wiady. U.S. Naval Medical Research Unit No 2, Jakarta Detachment, INDONESIA.
- 261 REACTIVITY OF SERA FROM A P. FALCIPARUM-P. VIVAX FOCUS IN INDONESIA WITH ERYTHROCYTE MEMBRANE ANTIGENS OF P. FALCIPARUM AND P. BRASILIANUM. A.J. Sulzer*, R.A. Cantella, M.D. Clarke, and W.P. Caney. Malaria Branch, Centers for Disease Control, Atlanta, GA; Departamento De Microbiologia, Universidad Peruana Cayetano Heredia, Lima, PERU; and Uniformed Services University of the Health Services, Bethesda, MD.
- 262 PRESENCE OF HISTIDINE RICH PROTEIN 2 (Pf HRP-2) IN THE SERA OF PEOPLE INFECTED WITH PLASMODIUM FALCIPARUM. M.E. Parra* and D.W. Taylor. Georgetown University, Washington, DC.
- 263 IDENTIFICATION OF MALARIAL ANTIGENS IN THE URINE OF INDIVIDUALS WITH PLASMODIUM FALCIPARUM INFECTIONS. M. Rodriguez-del Valle* and D.W. Taylor. Georgetown University, Washington, DC.
- 264 NEOPTERIN AS A QUANTITATIVE MEASURE OF CELLULAR IMMUNE RESPONSE DURING ACUTE FALCIPARUM MALARIA. A.E. Brown*, P. Teja-Isavadharm, D. Keeratithakul, and H.K. Webster. U.S. Army Medical Component, AFRIMS, Bangkok, THAILAND.
- 265 PREVALENCE OF NATURALLY ACQUIRED ANTIBODIES TO A 43 AMINO ACID PEPTIDE DERIVED FROM PLASMODIUM FALCIPARUM ERYTHROCYTE BINDING ANTIGEN 175. B.K.L. Sim*, H.C. Wynn, M. Carter, H. Marwoto, and S.L. Hoffman. Walter Reed Army Institute of Research, Washington, DC; Johns Hopkins University, Baltimore, MD; Naval Medical Research Institute, Bethesda, MD; and U.S. Naval Medical Research Unit No. 2 Detachment and National Institutes of Health Research and Development, Jakarta, INDONESIA.
- 266 ANTIBODY TO THE RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN (RESA): PREDICTIVE VALUE OF PROTECTION IN A RURAL COMMUNITY IN MADAGASCAR. P. Astagneau*, J.P. Lepers, C. Chougnet, C. Gaudebout, I. Hatin, E. Bailly, P. Coulanges, and P. Deloron. INSERM Unite, Paris, FRANCE and Institut Pasteur de Madagascar, Antananarivo, MADAGASCAR.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (MALARIA - BLOOD STAGES - Continued)

- 267 IDENTIFICATION OF A 50 KD TRYPSIN FRAGMENT FROM PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES THAT BINDS TO THE CD36 MALARIA SEQUESTRATION RECEPTOR. A.I. Meierovics, C.C. Magowan, N.N. Tandon, C.F. Ockenhouse, G.A. Jamieson, and J.D. Chulay. Walter Reed Army Institute of Research, Washington, DC.

CLINICAL TROPICAL MEDICINE

- 268 OUTBREAK OF ACUTE SCHISTOSOMIASIS IN A GROUP OF AMERICANS RETURNING FROM COTE D'IVOIRE. R. T. Bryan*, M.K. Michelson, M. Wilson, and S. Wahlquist. Centers for Disease Control, Atlanta, GA.
- 269 NEONATAL MORTALITY ASSOCIATED WITH LOW BIRTH WEIGHT IN MALAWI. L. Slutsker*, J.J. Wirima, C.O. Khoromana, and R.W. Steketee. Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA and Ministry of Health, Lilongwe, MALAWI.
- 270 SEVERITY OF ANEMIA IN ZAMBIAN CHILDREN WITH PLASMODIUM FALCIPARUM MALARIA. T.L. Fisk*, J.A. Wadje, I. Mbuze, E.B. Mvula, and G. Kakompe. Emory University School of Medicine, Atlanta, GA and Mukinge Hospital, Kasempa, ZAMBIA.
- 271 FETAL WASTAGE IN MALAWI, AN AREA OF HIGH MALARIA ENDEMICITY. J.G. Breman*, R.W. Steketee, J. Wirima, D.L. Heymann, and C. Khoromana. Centers for Disease Control, Atlanta, GA; Ministry of Health, Lilongwe, MALAWI.
- 272 CONGENITAL MALARIA: SPONTANEOUS POST-PARTUM CLEARANCE, MALAWI. J.J. Wirima*, D.L. Heymann, and R. Steketee. Ministry of Health, MALAWI; Centers for Disease Control, Atlanta, GA.
- 273 FEVER AND MALARIA IN HOSPITALIZED CHILDREN KINSHASA, ZAIRE. K. Hedberg*, N. Shaffer, F. Davachi, L. Bongo, K.M. Paluku, A. Vernon, P. Nguyen-Dinh, A.W. Hightower, and J.G. Breman. Centers for Disease Control, Atlanta, GA; Mama Yemo Hospital and PEV/CCCD, Kinshasa, ZAIRE.
- 274 ORAL CIPROFLOXACIN VERSUS CEFTRIAXONE FOR THE TREATMENT OF PENICILLIN-RESISTANT GONOCOCCAL URETHRITIS. J.P. Bryan*, S.K. Hira, W. Brady, C. Mwale, G. Mpoko, R. Krieg, E. Siwialiondo, C. Reichart, and P.L. Perine. Uniformed Services University of the Health Sciences, Bethesda, MD and University Teaching Hospital, Lusaka, ZAMBIA.
- 275 PRECOOKED RICE POWDER ORS COMPARED TO STANDARD CITRATE-GLUCOSE ORS IN MAINTENANCE THERAPY OF REHYDRATED HOSPITALIZED CHOLERA PATIENTS IN JAKARTA. N.H. Punjabi*, C. Rasidi, S. Sundah, S.P. Pulungsih, M.A. Mochtar, N. Sukri, D.H. Burr, N.D. Witham, and F.P. Paleologo. U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta, INDONESIA; Infectious Diseases Hospital, Jakarta, INDONESIA.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (CLINICAL TROPICAL MEDICINE - Continued)

- 276 DOUBLE BLIND CONTROLLED TRIAL TO DETERMINE SIDE EFFECTS AND IMMUNOGENICITY OF PARENTERAL VI CAPSULAR POLYSACCHARIDE (CPS) TYPHOID VACCINE IN INDONESIAN CHILDREN AND ADULTS. F.P. Paleologo*, C.H. Simanjuntak, N.H. Punjabi, S. Hardjining, T. Pudjarwoto, and N.D. Witham. U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta, INDONESIA; Ministry of Health, Jakarta, INDONESIA.
- 277 CHLOROQUINE DOES NOT AFFECT THE ANTIBODY RESPONSE TO YELLOW FEVER VACCINE. M. Barry, J. Patterson, B. Ratcliff, S. Tirrell, and R.E. Shope. Yale University School of Medicine, New Haven, CT.
- 278 DETECTION OF MALARIA IN POPULATIONS WITH LOW-DENSITY PARASITEMIAS. C. Wongsrichanalai*, J. Pornsilapatip, V. Namsiripongpun, H.K. Webster, A. Lucchini, H. Wilde, M. Prasittisuk, and S. Ketangsri. U.S. Army Medical Component, AFRIMS, Bangkok, THAILAND.
- 279 CHLOROQUINE-RESISTANT FALCIPARUM MALARIA ACQUIRED IN BURKINA FASO BY A BRITISH TRAVELER. M.S. Wolfe*, A.M.J. Oduola, D.E. Kyle, L. Gerena, L.C. Patchen, and W.K. Milhous. Medical Service, Department of State and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and the Centers for Disease Control, Atlanta, GA.
- 280 FECAL LEUKOCYTES: A SIMPLE MEANS FOR THE DIAGNOSIS AND MANAGEMENT OF DYSENTERY BY HEALTH CARE PROVIDERS IN EGYPT. N.A. El Masry*, S. Bassily, and Z. Farid. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT.
- 281 TUBERCULOUS LYMPHADENITIS IN CAIRO, EGYPT. Z. Farid*, M. Kamal, Y. Safwat, Z.A. Salama, and M. Kilpatrick. U.S. Naval Medical Research Unit No. 3; Abassia Fever Hospital, Ministry of Health, and Kasr El Aini Faculty of Medicine, Cairo University, Cairo, EGYPT.
- 282 DEXAMETHASONE IN BACTERIAL MENINGITIS. N.I. Girgis, Z. Farid, I.A. Mikhail, and R.L. Haberberger. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT.
- 283 EFFICACY OF MEFLOROQUINE AND CHLOROQUINE PROPHYLAXIS IN PREGNANCY. R.W. Steketee*, J. Wirima, D.L. Heymann, C. Khoromana, and J.G. Breman. Centers for Disease Control, Atlanta, GA; and Ministry of Health, Lilongwe, MALAWI.
- 284 PRAZIQUANTEL TREATMENT FOR SCHISTOSOMIASIS CONTROL IN QALUBIA GOVERNATE, EGYPT: 1976-1984. M.K. Michelson*, B.L. Cline, M.A. Habib, E. Ruiz-Tiben, F.M. Gamil, D.D. Juranek, and H.C. Spencer. Center for Disease Control, Atlanta, GA; Tulane University, New Orleans, LA; Center for Field and Applied Research, Ministry of Health, Cairo, EGYPT.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (Continued)

ARBOVIRAL ENTOMOLOGY

- 285 BIONOMICS OF CANOPY MOSQUITOES IN A TROPICAL FOREST. J.L. Petersen*. Gorgas Memorial Laboratory, PANAMA.
- 286 PERSISTENCE OF MOSQUITO-BORNE ARBOVIRUSES IN KERN COUNTY, CALIFORNIA, 1983-1987. W.K. Reisen, J.L. Hardy, W.C. Reeves, S.B. Presser, M.M. Milby, and R.P. Meyer. School of Public Health, University of California, Berkeley. Berkeley, CA.
- 287 MODELING THE EXTRINSIC INCUBATION OF ARBOVIRUSES IN MOSQUITOES IN KERN COUNTY CALIFORNIA. R.P. Meyer*, J.L. Hardy, and W.K. Reisen. School of Public Health, University of California, Berkeley, CA.
- 288 MONITORING ENVIRONMENTAL PARAMETERS ASSOCIATED WITH THE FLOODING OF RIFT VALLEY FEVER VIRUS VECTOR MOSQUITO HABITATS IN KENYA WITH POLAR ORBITING METEOROLOGICAL SATELLITE DATA. K.J. Linthicum, C.L. Bailey, C.J. Tucker, K.D. Mitchell, T.M. Logan, F.G. Davies, D.J. Dohm*, C.W. Kamau, P.C. Thande, and J.N. Wagathe. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; NASA, Goddard Space Flight Center, Greenbelt, MD; U.S. Naval Medical Research Unit, KENYA; Veterinary Research Laboratory, KENYA.
- 289 TRANSMISSION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY STRAINS OF Aedes albopictus COLLECTED IN NORTH AND SOUTH AMERICA. J.R. Beaman, and M.J. Turell. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 290 EFFECTS OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION ON THE BIOLOGY OF ITS ENZOOTIC MOSQUITO VECTOR, Culiseta melanura. T.W. Scott*, S.C. Weaver, and L.H. Lorenz. University of Maryland, College Park, MD.
- 291 MODULATION OF ALPHAVIRAL REPLICATION BY Aedes albopictus CELLS IN TISSUE CULTURE. E.J. Houk*, L.D. Kramer, J.L. Hardy, and S.B. Presser. School of Public Health, University of California, Berkeley, CA.
- 292 FURTHER CHARACTERIZATION OF THE Culex tarsalis MODEL FOR MODULATION OF WESTERN EQUINE ENCEPHALOMYELITIS (WEE) VIRUS. L.D. Kramer*, E.J. Houk, J.L. Hardy, and S.B. Presser. University of California, Berkeley, CA.
- 293 SUSCEPTIBILITY OF Aedes aegypti TO DENGUE-1 IN PANAMA. B.E. Dutary* and T. Solano. Gorgas Memorial Laboratory, PANAMA.
- 294 ENDOPHILIC BEHAVIOR OF Aedes aegypti IN PUERTO RICO. G.G. Clark*, H. Seda, and D.J. Gubler. Centers for Infectious Diseases, Centers for Disease Control, San Juan, PUERTO RICO.
- 295 LACROSSE VIRUS SMALL SEGMENT GENE EXPRESSION IN Aedes triseriatus MOSQUITO MIDGUTS. L.W. Wasieleski*, B.J. Beaty, L.J. Chandler, and M.J. Hewlett. Colorado State University, Fort Collins, CO and University of Arizona, Tucson, AZ.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (ARBOVIRAL ENTOMOLOGY - Continued)

- 296 CALIFORNIA AND BUNYAWERA SEROGROUP BUNYAVIRUSES FROM CALIFORNIA MOSQUITOES. G.L. Campbell*, B.F. Eldridge, J.L. Hardy, W.C. Reeves, and D.A. Dritz. University of California, Berkeley, CA and University of California, Davis, CA.
- 297 DETECTION OF RIFT VALLEY FEVER VIRUS RNA IN PARAFFIN SECTIONS OF MOSQUITOES BY IN SITU HYBRIDIZATION. L.A. Patrican* and W.S. Romoser. Ohio University, Athens, OH.
- 298 TRANSMISSION OF RIFT VALLEY FEVER VIRUS BY ADULT MOSQUITOES AFTER INGESTION OF VIRUS WHILE LARVAE. M.J. Turell, K.J. Linthicum, and J.R. Beaman. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 299 NONVASCULAR DELIVERY OF RIFT VALLEY FEVER VIRUS BY INFECTED MOSQUITOES. M.J. Turell, A. Spielman, and R.A. Tammariello. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, and Harvard School of Public Health, Boston, MA.
- 300 THE USE OF MOLECULAR GENETIC MARKERS TO ANALYZE POPULATION GENETIC STRUCTURE OF THE VECTOR OF BLUETONGUE VIRUS, CULICOIDES VARIIPENNIS. W.J. Tabachnick* and W.C. Wilson. U.S. Department of Agriculture, Agricultural Research Service, Arthropod-Borne Animal Diseases Research Laboratory, Laramie, WY.
- 301 ISOLATION OF VIRUSES FROM CULICOIDES MIDGES DURING AN EPIZOOTIC OF VESICULAR STOMATITIS NEW JERSEY, 1982. W.L. Kramer, R.H. Jones, F.R. Holbrook, T.E. Walton, and C.H. Calisher. Nebraska Department of Health, Lincoln, NE; Arthropod-Borne Animal Diseases Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Laramie, WY; and Centers for Disease Control, Fort Collins, CO.

VIRAL EPIDEMIOLOGY

- 302 PREVALENCE OF ANTIBODIES TO FLAVIVIRUSES, SANDFLY VIRUSES AND LEPTOSPIROSIS IN FEVER PATIENTS AT THE MILITARY HOSPITAL, RAWALPINDI, PAKISTAN. R.E. Krieg*, J.F. Duncan, J.P. Bryan, T.G. Ksiazek, J.W. LeDuc, B. Awan, A. Ahmed, M. Raiz, S. Nabi, P.L. Perine, L.L. Legters, M. Iqbal, and I.A. Malik. Uniformed Services University of the Health Sciences, Bethesda, MD; PULSE, AMC, Rawalpindi, PAKISTAN; and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick MD.
- 303 SEROLOGIC PREVALENCE OF ARBOVIRUSES IN WHITE-TAILED DEER IN SOUTH FLORIDA, 1984-1988. R.G. McLean*, S.D. Wright, S.R. Ubico, and D.J. Forrester. Centers for Disease Control, Fort Collins, CO and University of Florida, Gainesville, FL.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (VIRAL EPIDEMIOLOGY - Continued)

- 304 CHIKUNGUNYA VIRUS INFECTIONS IN PATIENTS AT SUMBER WARAS HOSPITAL, JAKARTA, INDONESIA, 1987-1988. C. Bartz, R. Tan, C. Maroef, A. Sie, H. Wulur, and T.K. Samsi. U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, Jakarta, INDONESIA, and Sumber Waras Hospital Jakarta, INDONESIA.
- 305 IDENTIFICATION OF EPIDEMIC CHIKUNGUNYA (CHIK) IN NORTHEASTERN THAILAND. A. Nisalak*, A. Srisajjakul, K. Nakdi, S. Rojanasuphot, C. Hemachudha, N. Nutkumhang, N. Sahasakmontri, and B. Innis. Armed Forces Research Institute of Medical Sciences, Bangkok and Division of Epidemiology and Department of Medical Sciences, Ministry of Public Health, THAILAND.
- 306 DENGUE FEVER: A REPORT ON THREE LABORATORY ACQUIRED INFECTIONS AT DIFFERENT INSTITUTIONS DURING 1988. M.P. Kiley, L.M. Alderman, J.W. McVicar, R.B. Craven, A.K. Galloway, F.J. Malinoski, and P.H. Hausser. Centers for Disease Control, Atlanta, GA and Fort Collins, CO; U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; and Yale University, New Haven, CT.
- 307 RECENT ISOLATIONS OF PUNTA TORO VIRUS FROM FEBRILE HUMANS IN PANAMA. J.A. Mangiafico*, J.L. Sanchez, D. Pifat, and J.W. LeDuc. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; and Walter Reed Army Institute of Research, Washington, DC.
- 308 PHLEBOVIRUS INFECTIONS AMONG HUMANS WITH UNDIFFERENTIATED ACUTE FEBRILE ILLNESS, EGYPT, 1988-1989. M. Darwish*, A. Zaki, D.M. Watts, T.G. Ksiazek, and C.J. Peters. Ain Shams University, Cairo, EGYPT; United States Naval Medical Research Unit No. 3, Cairo, EGYPT; and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 309 VIROLOGICAL AND SEROLOGICAL RESULTS FROM NATURAL AND EXPERIMENTAL INFECTIONS BY CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN WEST AFRICA. J.P. Gonzalez*, B. LeGuanno, M. Guillaud, J.P. Cornet, J.L. Camicas, J.P. Digoutte, and M.L. Wison. Institut Pasteur, Dakar, SENEGAL; Institut d'Elevage et de Medecine Veterinaire des Pays Tropicaux; Laboratoire ORSTOM de Zoologie Medicale; U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 310 PREVALENCE OF JUNIN VIRUS (JV) ANTIGEN AND ANTIBODY AMONG RODENTS IN ENDEMIC AND NON-ENDEMIC AREAS OF ARGENTINE HEMORRHAGIC FEVER (AHF). J. Mills, B. Ellis, J. Maizteguo, T. Ksiazek, K. McKee*, and J. Childs. Johns Hopkins University, Baltimore, MD; INEVH, Pergamino, ARGENTINA, and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 311 LIMITED PROTECTION AGAINST RABIES VIRUS CONFERRED BY IMMUNIZING MICE WITH CERTAIN OTHER RHABDOVIRUSES. C.H. Calisher*, N. Karabatsos, L. Thompson, and J.S. Smith. Centers for Disease Control, Fort Collins, CO and Atlanta, GA; and U.S. Department of Agriculture, Agricultural Research Service, Laramie, WY.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (VIRAL EPIDEMIOLOGY - Continued)

- 312 DECLINING HEPATITIS A ANTIBODY PREVALENCE AMONG CHILDREN IN THAILAND. B.L. Innis, R. Snitbhan*, T. Laorakpongse, W. Munindhorn, S. Sriprapandh, and C.H. Hoke. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok and Ministry of Public Health, THAILAND.

EPIDEMIOLOGY

- 313 A WATERBORNE OUTBREAK OF LEPTOSPIROSIS AMONG U.S. MILITARY PERSONNEL IN OKINAWA, JAPAN. A. Corwin*, A. Ryan, W. Bloys, R. Thomas, B. Deniega, and D. Watts. 3rd Marine Division, FMFPAC, Okinawa, JAPAN, U.S. Navy Environmental and Preventive Medicine Unit No. 6, Pearl Harbor, HI; Walter Reed Army Institute of Research, Washington, DC; and U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT.
- 314 TIBERIAN ENVIRONMENT AND INFECTIONS: ANTIBODIES AGAINST HANTAVIRUS, LEPTOSPIRES, AND HEPATITIS A VIRUS IN EXPOSED PERSONS ON THE RIVER BANKS IN ROME. M. Nuti*, D. Amaddeo, G. Antoniazzi, E. Franco, and C. Lalli. University 'La Sapienza', Institute of Zooprofilaxis, St. Anna Hospital, Department of Public Health, University 'Tor Vergata', and Police Medical Service, Rome, ITALY.
- 315 EPIDEMIC KERATOCONJUNCTIVITIS AT A U.S. MILITARY BASE: REPUBLIC OF THE PHILIPPINES. S.F. Paparello*, L.S. Rickman, H.N. Nesbahi, J.B. Ward, and C.G. Hayes. National Naval Medical Center, Bethesda, MD; 13th Air Force Medical Center and Naval Medical Research Institute Unit No. 2, Manila, THE PHILIPPINES.
- 316 THE RISK OF VIBRIO INFECTION AMONG RAW OYSTER CONSUMERS IN FLORIDA. J.C. Desenclos*, K.A. Klontz. DFS/EPO/CDC, Department of Health and Rehabilitative Services, Tallahassee, FL.
- 317 BACTERIAL PATHOGENS ASSOCIATED WITH INFECTIOUS DIARRHEA IN DJIBOUTI. I.A. Mikhail*, R.L. Haberberger, E. Fox, E.A. Abbatte, and M. Ahmed. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT; International Health Program, University of Maryland School of Medicine, Baltimore, MD; and Ministry of Public Health, DJIBOUTI.
- 318 EPIDEMIOLOGY OF HYDATIDOSIS IN THE WEST BANK: A RETROSPECTIVE STUDY. M.I. Othman*, M. Abdual Shafi, N. Abu-Hasan, and G.I. Higashi. Naja University, Nablus, West Bank, Makassed Hospital, Jerusalem, ISRAEL, and University of Michigan, Ann Arbor, MI.
- 319 AN UNPRECEDENTED EPIDEMIC OF MALARIA IN THE REPUBLIC OF DJIBOUTI DURING THE WINTER SEASON 1988/89. E. Fox*, E.A. Abbatte, H.H. Wassef, E. Mikhail, N.T. Constantine, D.M. Watts, M.A. Goulam, and C. Bailly. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT; International Health Program, University of Maryland School of Medicine, Baltimore, MD; Ministry of Public Health, DJIBOUTI; and WHO/EMRO.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (EPIDEMIOLOGY - Continued)

- 320 IMPORTED INTESTINAL PARASITE INFECTIONS: PREVALENCE IN CAMBODIAN REFUGEES SIX YEARS AFTER ARRIVAL IN CANADA. T.W. Gyorkos*, J.D. MacLean, P. Viens, and C.G. Law. Division of Clinical Epidemiology and McGill University Centre for Tropical Disease, Montreal General Hospital, Montreal, and Centre de Cooperation Internationale en sante et developement, Universite Laval, Quebec, CANADA.

CRYPTOSPORIDIUM

- 321 CELLULAR IMMUNE RESPONSES IN THE SMALL INTESTINE OF BALB/c MICE INFECTED WITH CRYPTOSPORIDIUM PARVUM. S.M. Novak*, M.J. Arrowood, and C.R. Sterling. University of Arizona, Tucson, AZ.
- 322 CRYPTOSPORIDIUM PARVUM INFECTION IN CHILDREN OF MEXICO CITY. F.J. Enriquez*, C. Avila, O. Vallejo, J. Tanaka, C. Alpuche, L.E. Espinoza, M.L. Ramirez, J.I. Santos, and C.R. Sterling. Division of Biotechnology, ARL, University of Arizona, Tucson, AZ; Department of Intestinal Bacteriology, Hospital Infantil de Mexico, Mexico City, MEXICO; and Direccion General de Epidemiologia, Ministry of Health, Mexico City, MEXICO.
- 323 IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF CRYPTOSPORIDIUM ANTIGENIC SITES AND AN ASSESSMENT OF THE ROLE OF MONOCLONAL ANTIBODIES IN CONTROLLING CRYPTOSPORIDIOSIS. M.H. Cho*, M.J. Arrowood, J.R. Mead, and C.R. Sterling. University of Arizona, Tucson, AZ.
- 324 CRYPTOSPORIDIUM MURIS-LIKE OBJECTS FROM FECAL SAMPLES OF PERUVIANS. J. Narango, C. Sterling*, R. Gilman, E. Miranda, F. Diaz, M. Cho, and A. Benel. Universidad Peruana Cayetano Heredia, Lima, PERU, University of Arizona, Tucson, AZ and The Johns Hopkins University, Baltimore, MD.
- 325 STATISTICAL ANALYSIS OF CLINICAL, IMMUNOLOGICAL, AND NUTRITIONAL FACTORS IN PEDIATRIC CRYPTOSPORIDIOSIS IN THE PHILIPPINES. D.M. Menorca, M.A. Laxer, A.K. Alcantara, M. Javato Laxer, M.T. Fernando, and V. Gonzales. U.S. NAMRU-2, and San Lazaro Hospital, Manila, THE PHILIPPINES.
- 326 SWIMMING-ASSOCIATED CRYPTOSPORIDIOSIS. F. Sorvillo, K. Fujioka, W. Tormey, R. Kebajian, W. Tokushige, and L. Mascola. Los Angeles County Department of Health Services, Los Angeles, CA.

TOXOPLASMA AND PNEUMOCYSTIS

- 327 A PROSPECTIVE STUDY OF THE TRANSMISSION OF TOXOPLASMA IN PANAMA. J.K. Frenkel*, R. Saenz, R. Quintero N., L.M. De Moreno, R. Centeno, L.O. Perez, R.J. Peart, M. Sousa, R. Galastica, E. Salas de Ortega, S.L. De Lao, and G.G. de Paredes. University of Kansas School of Medicine, Kansas City, KS, and Gorgas Memorial Laboratory, Panama City, PANAMA.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (TOXOPLASMA AND PNEUMOCYSTIS - Continued)

- 328 DIVERSITY OF rRNA SPECIES AND STRAINS OF PNEUMOCYSTIS CARINII. J.S. Shah*, A. Buharin, and D. Lane. GENE-TRAK Systems, Framingham, MA.
- 329 PNEUMOCYSTIS AND TOXOPLASMA DIHYDROFOLATE REDUCTASES USED IN DRUG SCREENING: ACTIVITIES OF PYRIMETHAMINE ANALOGS. M.C. Broughton, M.S. Bartlett, and S.F. Queener.* Indiana University School of Medicine, Indianapolis, IN.
- 330 FATTY ACID METABOLISM OF PNEUMOCYSTIS CARINII IN CULTURE. J.R. Paulsrud*, T.P. Glancy, M.M. Shaw, M.S. Bartlett, and J.W. Smith. Indiana University School of Medicine, Indianapolis, IN.

RETROVIRAL INFECTIONS

- 331 COMPARISON OF PARTICULATE 3,3',5,5'-TETRAMETHYLBENZIDINE AND 3,3'-DIAMINOBENZIDINE AS CHROMOGENIC SUBSTRATES FOR IMMUNOBLOT. J.A. Brand, V.C.W. Tsang, W. Zhou*, and S.B. Shukla. Centers for Disease Control, Atlanta, GA, and Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD.
- 332 RETROVIRAL SURVEILLANCE IN SOMALIA. D.A. Scott*, A. Corwin, M.A. Omar, A. Guled, N. Constantine and D. Watts. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT and Ministry of Health, SOMALIA.
- 333 HTLV-I INFECTION AMONG BLOOD DONORS, CHILDREN, AND HIGH RISK GROUPS IN THE MIDDLE EAST. N.T. Constantine*, A.L. Corwin, D.A. Scott, M.A. Mohamed, N.M. Osman, and D.M. Watts. U.S. Medical Research Unit No. 3 and Al Azhar University, Cairo, EGYPT.

MALARIA - CHEMOTHERAPY

- 334 MECHANISMS RESPONSIBLE FOR THE DESTRUCTION OF PLASMODIUM FALCIPARUM BY ASCORBIC ACID AND COPPER. J. Golenser, E. Marva, A. Cohen, and M. Chevion. The Hebrew University, Jerusalem, ISRAEL.
- 335 ANTIMALARIAL ACTIVITY OF PEPSTATIN A AND E 64 ON P. FALCIPARUM AND P. YOELII. E. Bailly, P. Deloron, I. Hatin, J. Savel, and G. Jaureguiberry, INSERM U13, Hospital Claude Bernard, Paris, FRANCE.
- 336 MEFLOQUINE CONCENTRATION IN WHOLE BLOOD AND URINE, AND ADVERSE DRUG REACTIONS IN HEALTHY VOLUNTEERS GIVEN A THERAPEUTIC DOSE OF MEFLOQUINE. L. Patchen*, and S. Williams. Centers for Disease Control, Atlanta, GA.
- 337 TREATMENT FOR AFRICAN CHILDREN WITH PLASMODIUM FALCIPARUM MALARIA IN AREAS OF CHLOROQUINE RESISTANCE: A COST-EFFECTIVENESS ANALYSIS. P. Sudre*, J.G. Breman, and D. McFarland. Centers for Disease Control, Atlanta, GA.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (MALARIA - CHEMOTHERAPY - Continued)

- 338 COMPARISON OF THE MULTIPLE DOSE KINETICS AND IN VITRO CHARACTERISTICS OF DAPSONE PLUS PROGUANIL VERSUS MALOPRIM. M.D. Edstein*, J.R. Veenendaal, and K.H. Rieckmann. Army Malaria Research Unit, Milpo, Ingelburn, NSW, AUSTRALIA.
- 339 PHARMACOKINETICS AND METABOLISM OF ARTEETHER IN THE ISOLATED PERFUSED RAT LIVER. P.R. McNally, A.D. Theoharides, J.O. Peggins, B.G. Schuster, and T.G. Brewer*. Walter Reed Army Institute of Research, Washington, DC.
- 340 IN VITRO INDUCTION OF RESISTANCE TO HALOFANTRINE IN CLONES OF PLASMODIUM FALCIPARUM. L. Gerena*, A.M.J. Oduola, B.G. Schuster, W.K. Milhous, and D.E. Kyle. Walter Reed Army Institute of Research, Washington, DC.
- 341 IN VITRO EFFECTS OF PRIMAQUINE AND PRIMAQUINE METABOLITES ON EXOERYTHROCYTIC DEVELOPMENT OF PLASMODIUM BERGHEI. M.D. Bates, S.R. Meshnick, C.I. Sigler, P. Leland, D. Hayes, and M.R. Hollingdale. Biomedical Research Institute, Rockville, MD; City University of New York Medical School, New York, NY.
- 342 GENETIC LINKAGE OF CHLOROQUINE RESISTANCE, RAPID CHLOROQUINE EFFLUX, REDUCED CHLOROQUINE ACCUMULATION AND VERAPAMIL REVERSAL IN PLASMODIUM FALCIPARUM. I.Y. Gluzman*, D.J. Krogstad, L.J. Panton, and T.E. Wellems. Washington University School of Medicine, St. Louis, MO, and National Institutes of Health, Bethesda, MD.
- 343 ACCUMULATION OF CHLOROQUINE BY MEMBRANE PREPARATIONS FROM PLASMODIUM FALCIPARUM. B.L. Herwaldt*, P.H. Schlesinger, and D.J. Krogstad. Washington University, St. Louis, MO.

ENTOMOLOGY

- 344 SPERM COMPETITION IN THE DEER TICK IXODES DAMMINI: PROSPECTS FOR CONTROL BY STERILE MALE RELEASE. B. Yuval and A. Spielman. Harvard School of Public Health, Boston, MA.
- 345 EVALUATION OF BARRIER SPRAYING FOR THE CONTROL OF MALARIA IN THE DOMINICAN REPUBLIC. M.J. Perich*, M.A. Tidwell, M.R. Sardelis, D.C. Williams, C.J. Pena, and L.R. Boobar. U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD; Vector Contro Project Universidad Catolica Madre Maestra, Santiago, DOMINICAN REPUBLIC; and International Center for Public Health Research McClellanville, SC.
- 346 TOXICITY EVALUATION OF METHOPRENE TO MOSQUITO LARVAE AND NONTARGET ORGANISMS. S. Sriharan*, T.P. Sriharan, and M. Thompson. Selma University, Selma, AL.
- 347 BIONOMICS OF CANOPY MOSQUITOES IN A TROPICAL FOREST. J.L. Petersen*. Gorgas Memorial Laboratory, PANAMA.

WEDNESDAY MORNING, DECEMBER 13

POSTER SESSION II (ENTOMOLOGY - Continued)

- 348 VARIATION IN CUTICULAR HYDROCARBON PROFILES OF NORTH AMERICAN AEDES ALBOPICTUS POPULATIONS. E.L. Kruger*, and C.D. Pappas. Peru State College, Peru, NE.
- 349 SPECIES DIVERSITY, BITING ACTIVITY AND FLAGELLATE INFECTION RATES OF PHLEBOTOMINE SAND FLIES COLLECTED AT TEKA, GUATEMALA. E.D. Rowton*, C.H. Porter, R.G. Andre, T.R. Navin, and J.L. Pozuelos. Walter Reed Army Institute of Research, Washington, DC; Centers for Disease Control, Atlanta, GA; Uniform Services University of Health Sciences, Bethesda, MD; Medical Entomology Research and Training Unit, Guatemala City, GUATEMALA.
- 350 FEMALE-SPECIFIC GENE EXPRESSION IN THE SALIVARY GLANDS OF MOSQUITOES. A.A. James*, K. Blackmer, O. Marinotti, and G. Grossman. Harvard School of Public Health, Boston, MA.
- 351 RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN MIDDLE REPETITIVE DNA OF AEDES ALBOPICTUS DETECTED BY SOUTHERN BLOT HYBRIDIZATION. T.J. Monroe*, J.O. Carlson, and R.J. Beaty. Colorado State University, Fort Collins, CO.

SCIENTIFIC SESSION M: LATE BREAKERS IN MOLECULAR BIOLOGY WORKSHOP

10:00 AM - 12:00 Noon

Coral III

Chairpersons: M.R. Hollingdale and S.R. Meshnick

Persons interested in presenting at this session should contact Dr. Hollingdale (301-881-3300) or Dr. Meshnick (301-2690-6628) within 3 weeks of the meeting or before noon on Monday, December 11. A list of the abstracts selected for presentation will be available in the Registration Area Bulletin Board beginning at 8:00 AM Tuesday, December 12.

Abstract

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SCIENTIFIC SESSION N: MOLECULAR VIROLOGY

10:00 AM - 12:00 Noon

Coral IV

Chairpersons: D.H.L. Bishop and R.M. Kinney

Time Abstract

- 10:00 360 GENOMIC AND ANTIGENIC COMPARISONS OF EASTERN EQUINE ENCEPHALITIS VIRUSES SUGGEST DIFFERENTIAL EVOLUTION OF NORTH AND SOUTH AMERICAN STRAINS. P.M. Repik*, and J.M. Strizki. The Medical College of Pennsylvania, Philadelphia, PA.

WEDNESDAY MORNING, DECEMBER 13

SCIENTIFIC SESSION N: MOLECULAR VIROLOGY (Continued)

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| 10:15 | 361 | YELLOW FEVER VIRUS EVOLUTION: COMPARATIVE ANALYSIS OF THE NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCES OF THE STRUCTURAL GENES OF TWO GEOGRAPHICALLY ISOLATED STRAINS. M.E. Ballinger* and B.R. Miller. Centers for Disease Control, Fort Collins, CO. |
| 10:30 | 362 | GENETIC RELATIONSHIP AMONG JAPANESE ENCEPHALITIS VIRUS STRAINS AS DETERMINED BY PRIMER-EXTENSION SEQUENCING. W.R. Chen*, R. Rico-Hesse, and R.B. Tesh. Yale University School of Medicine, New Haven, CT. |
| 10:45 | 363 | GENETIC ANALYSIS OF VARIATION IN THE NUCLEOTIDE SEQUENCES AND DEDUCED AMINO ACID SEQUENCES OF THE STRUCTURAL AND NS-1 NON-STRUCTURAL GENES FROM DENGUE 1 (16007) AND EARLY PRIMARY DOG KIDNEY PASSAGE STRAINS. M.C. Chu*, R. Putvatana, and D.W. Trent. Centers for Disease Control, Fort Collins, CO. |
| 11:00 | 364 | EXPRESSION OF THE DENGUE-2 ENVELOPE GLYCOPROTEIN IN MAMMALIAN CELLS. E.P. Kelly*, R.J. Feighny, and C.H. Hoke. Walter Reed Army Institute of Research, Washington, DC. |
| 11:15 | 365 | RESPONSE IN MICE FOLLOWING INOCULATION WITH DENGUE-2 PROTEINS ISOLATED BY HIGH PRESSURE LIQUID CHROMATOGRAPHY. R.J. Feighny*, M.J. Burrous, J.M. McCown, J.R. Putnak, and C.H. Hoke. Walter Reed Army Institute of Research, Washington, DC. |
| 11:30 | 366 | SYNTHETIC PEPTIDES DERIVED FROM THE E-GLYCOPROTEIN OF DENGUE 2 VIRUS DEFINE ANTIGENIC AND STRUCTURAL CHARACTERISTICS. J.T. Roehrig*, A.R. Hunt, A.J. Johnson, R.A. Bolin, and M.C. Chu. Centers for Disease Control, Fort Collins, CO. |
| 11:45 | 367 | THE COMPLETE SEQUENCE OF VP5 OF BROADHAVEN VIRUS, A KEMEROVO SERO-GROUP ORBIVIRUS, AND ITS RELATIONSHIP TO BLUETONGUE VIRUS. S.R. Moss and P.A. Nuttall. NERC Institute of Virology and Environmental Microbiology, Oxford, UK. |

SCIENTIFIC SESSION O: ENTOMOLOGY - LYME DISEASE

10:30 AM - 1:00 Noon

South Pacific I - II

Chairperson: A. Spielman

Time Abstract

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| 10:30 | 363 | NATIONAL SURVEILLANCE OF LYME DISEASE, 1987-1988. T.F. Tsai*, R.E. Bailey, and G.W. Letson. Centers for Disease Control, Fort Collins, CO. |
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WEDNESDAY MORNING, DECEMBER 13

SCIENTIFIC SESSION O: ENTOMOLOGY - LYME DISEASE (Continued)

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| 10:45 | 369 | GROWTH KINETICS OF THE LYME DISEASE SPIROCHETE IN IXODES TICKS. J. Piesman*, J.R. Oliver, and R.J. Sinsky. University of Alabama at Birmingham, Birmingham, AL. |
| 11:00 | 370 | LYME DISEASE IN IOWA. A.M.G. Novak, K.B. Platt, and W.A. Rowley*. Iowa State University, Ames, IA. |
| 11:15 | 371 | FACTORS STIMULATING IXODES-BORNE PATHOGENS TO MATURE. S.R. Telford, III*, and A. Spielman. Harvard School of Public Health, Boston, MA. |
| 11:30 | 372 | INVASIVENESS OF THE LYME DISEASE SPIROCHETE UNRELATED TO HYALURONIDASE ACTIVITY. R.B. Kimsey, R.J. Pollack*, and A. Spielman. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA. |
| 11:45 | 373 | LACK OF LYME DISEASE SPIROCHETE TRANSMISSION FROM RESERVOIR MICE (<u>PEROMYSCUS LEUCOPUS</u>) TO THEIR OFFSPRING. T.N. Mather*, S.R. Telford, III, and G.H. Adler. Harvard School of Public Health, Boston, MA. |

SCIENTIFIC SESSION P: AMEBIASIS

10:00 AM - 12:00 Noon

South Pacific III - IV

Chairpersons: K. Chadee and R.A. Salata

Time Abstract

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| 10:00 | 374 | A MONOCLONAL ANTIBODY SPECIFIC FOR ELECTRON-DENSE GRANULES SECRETED BY <u>ENTAMOEBA HISTOLYTICA</u> . M.L. Munoz*, J.N. P. Garcia, M. de la Torre, G.R. Tovar, and G. Leon. Centro de Investigacion y de Estudios Avanzados, del IPN; IMSS, D.F. MEXICO. |
| 10:15 | 375 | INHIBITION OF IN VITRO CYTOTOXICITY AND ION CHANNEL FORMING ACTIVITY OF <u>ENTAMOEBA HISTOLYTICA</u> BY MURINE MONOCLONAL ANTIBODIES. J.N. Aucott*, I.J. Malholtra, and R.A. Salata. Case Western Reserve University and University Hospitals, Cleveland, OH. |
| 10:30 | 376 | CYTOKINE ACTIVATED HUMAN NEUTROPHILS KILL <u>ENTAMOEBA HISTOLYTICA</u> TROPHOZOITES IN VITRO. K. Chadee* and M. Denis. Institute of Parasitology of McGill University, Ste-Anne-de-Bellevue, Quebec, CANADA. |
| 10:45 | 377 | IDENTIFICATION OF THE FIBRONECTIN RECEPTOR OF <u>ENTAMOEBA HISTOLYTICA</u> AS THE 35 KD SUBUNIT OF THE GAL/GALNAC ADHERENCE LECTIN. B.J. Mann* and W.A. Petri, Jr. University of Virginia, Charlottesville, VA. |

WEDNESDAY MORNING, DECEMBER 13

SCIENTIFIC SESSION P: AMEBIASIS (Continued)

- 11:00 378 THE GALACTOSE AND N-ACETYL-D-GALACTOSAMINE (GAL/GALNAc) ADHERENCE LECTIN OF ENTAMOEBA HISTOLYTICA IS DETECTED BY RIA ONLY IN PATHOGENIC STRAINS. W.A. Petri, Jr.*, T.F.H.G. Jackson, and D. Mirelman. University of Virginia, Charlottesville, VA; Research Institute for Diseases in a Tropical Environment, Durban, SOUTH AFRICA; and Weizmann Institute, Rehovot, ISRAEL.
- 11:15 379 SERUM ANTI-ADHERENCE LECTIN ANTIBODIES AS A MARKER OF PATHOGENIC ENTAMOEBA HISTOLYTICA INFECTION. J.I. Ravdin*, T.F.H.G. Jackson, W.A. Petri, Jr., C.F. Murphy, B.L.P. Ungar, V. Gathiram, J. Skilogiannis, and A.E. Simjee. University of Virginia, Charlottesville, VA; Research Institute for Diseases in a Tropical Environment, SOUTH AFRICA; Uniformed Health Services University, Bethesda, MD; University of Natal, SOUTH AFRICA.
- 11:30 380 ANTIGENICITY OF DEGLYCOSYLATED ENTAMOEBA HISTOLYTICA PROTEINS. E.L.W. Kittler* and J.I. Ravdin. University of Virginia School of Medicine, Charlottesville, VA.
- 11:45 381 IDENTIFICATION OF A SURFACE MOLECULE OF ENTAMOEBA HISTOLYTICA WITH IMMUNODOMINANT CHARACTERISTICS USING SERA FROM PATIENTS WITH HEPATIC ABSCESS. M.A. Meraz*, U. Edman, N. Agabian, and I. Meza. University of California, San Francisco, CA.

SCIENTIFIC SESSION Q: TAPEWORMS

9:45 AM - 12:15 PM

Nautilus II - III

Chairpersons: P.M. Schantz and A. Flisser

Time Abstract

- 9:45 382 EVALUATION OF A HYDATID DISEASE CONTROL PROGRAM IN THE XINJIANG/UYGUR AUTONOMOUS REGION, PRC (CHINA). F.L. Andersen*, H.D. Tolley, P.M. Schantz, P. Chi, F. Liu, and Z. Ding. Brigham Young University, Provo, UT; Centers for Disease Control, Atlanta, GA; Veterinary Research Institute, National Hydatid Disease Center of China and Xinjiang Medical College, Urumqi, Xinjiang, PEOPLE'S REPUBLIC OF CHINA.
- 10:00 383 COUNTERIMMUNOELECTROPHORESIS USING AN ARC 5 ANTIGEN IS SPECIFIC FOR DIAGNOSIS OF CYSTIC HYDATID DISEASE. P.R. Mira*, H. Shweiki, I. Francis, and K. Behbehani. Faculty of Medicine, Kuwait University, Safat, KUWAIT.

WEDNESDAY MORNING, DECEMBER 13

SCIENTIFIC SESSION Q: TAPEWORMS (Continued)

- 10:15 384 ALBENDAZOLE THERAPY OF ECHINOCOCCUS MULTILOCULARIS INFECTION IN THE MONGOLIAN JIRD (MERIONES UNGUICULATUS). P.M. Schantz*, F.H. Brandt, C.M. Dickinson, C.R. Allen, J.M. Roberts, and M.L. Eberhard. Centers for Disease Control, Atlanta, GA.
- 10:30 385 CYSTICERCOSIS SURVEILLANCE - LOS ANGELES COUNTY. F.J. Sorvillo*, S.H. Waterman, F.O. Richards, and P.M. Schantz. Los Angeles County Department of Health Services, Los Angeles, CA; and the Centers for Disease Control, Atlanta, GA.
- 10:45 386 THE EXAMINATION OF THE TONGUE; A RAPID METHOD FOR THE DIAGNOSIS OF PORCINE CYSTICERCOSIS COMPARED TO SEROLOGY USING THE ELISA OR IMMUNOBLOT ASSAY. E.A. Gonzalez*, V. Cama, R.H. Gilman, V. Tsang, J. Brandt, E. Miranda, T. Montenegro and M. Verastigui. Universidad de San Marcos, PRISMA, Universidad de Cayetano Heredia; John Hopkins University, Baltimore, MD; and Centers for Disease Control, Atlanta, GA.
- 11:00 387 SERO-EPIDEMIOLOGY OF CYSTICERCOSIS OF A RURAL VILLAGE IN PERU USING THE IMMUNOBLOT ASSAY. R.H. Gilman*. The Cysticercosis Working Group in Peru and the Centers for Disease Control; UPCH, Lima, PERU.
- 11:15 388 SEROLOGICAL DIAGNOSIS OF CYSTICERCOSIS - COMPARISON OF THE ELISA ASSAY FOR ANTIBODY (EAB) AND ANTIGEN DETECTION (EAG) COMPARED TO THE ENZYME IMMUNO-TRANSFER BLOT (EITB) TEST. D. Diaz*. The Cysticercosis Working Group in Peru and the Centers for Disease Control; UPCH, Lima, PERU.
- 11:30 389 IMMUNOBLOT ASSAY OF ANTIBODY RESPONSES IN PIGS WITH CYSTICERCOSIS FROM NATURAL INFECTIONS AND MODULATED ISOTYPIC ACTIVITIES IN EXPERIMENTAL ANIMALS. V.C.W. Tsang*, J.A. Brandt, W. Zhou, A.E. Boyer, E.I.P. Kammago-Sollo, M. Rhoads, D. Murrell, P.M. Schantz, and R.H. Gilman. Centers for Disease Control, Atlanta, GA; Agricultural Research Service, Beltsville, MD; and U. Peruana Cayetano Heredia, Lima, PERU.
- 11:45 390 EFFECT OF PRAZIQUANTEL ON TAENIA SOLIUM CYSTICERCI IN VITRO. C. Garcia-Dominguez, D. Correa, M.T. Rabiela, and A. Flisser*. Instituto de Investigaciones Biomedicas. Instituto Nacional de Diagnostico y Referencia Epidemiologicos: 'Dr. Manuel Martinez Baez', SSA., MEXICO.
- 12:00 391 REPETITIVE DNA SEQUENCES IN TAENIA SOLIUM AND TAENIA SAGINATA. V. Vallejo, A. Fauconnier, J.P. Lacleste, E. Garcia-Zepeda, D.P. McManus, and A. Flisser. Instituto de Investigaciones Biomedicas, UNAM, MEXICO; Queensland Institute of Medical Research, Brisbane, AUSTRALIA.

WEDNESDAY AFTERNOON - DECEMBER 13

SCIENTIFIC SESSION R: FILARIASIS - ONCHOCERCIASIS AND IVERMECTIN

1:30 PM - 5:00 PM

Coral III

Chairpersons: M. Eberhard and F. Richards

Time Abstract

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| 1:30 | 392 | IMPROVED METHOD FOR EXAMINING THE STRUCTURE AND REPRODUCTIVE STATUS OF <u>ONCHOCERCA VOLVULUS</u> . B.O.L. Duke. Armed Forces Institute of Pathology, Washington, DC. |
| 1:45 | 393 | INITIATION OF VERTEBRATE-PHASE DEVELOPMENT BY LARVAL FILARIAE: ROLES OF TEMPERATURE AND MAMMALIAN SERUM IN DEVELOPMENTAL ACTIVATION. K.M. Lee, M.J. Balsai and J.B. Lok*. University of Pennsylvania, Philadelphia, PA. |
| 2:00 | 394 | EXPERIMENTAL <u>ONCHOCERCA VOLVULUS</u> INFECTION IN A MANGABEY MONKEY (<u>CERCOCEBUS ATYS</u>): PARASITOLOGICAL AND IMMUNOLOGICAL OBSERVATIONS. M. Eberhard*, A. Boyer, J. Dickerson, V. Tsang, F. Richards, R. Zea-Flores, E. Walker, and G. Zea-Flores. Centers for Disease Control, Atlanta, GA; Emory University, Atlanta, GA; and Ministry of Health, GUATEMALA. |
| 2:15 | 395 | ISOLATION AND CHARACTERIZATION OF EXPRESSION cDNA CLONES ENCODING ANTIGENS OF <u>ONCHOCERCA VOLVULUS</u> . S. Lustingman and A.M. Prince. The Lindsley F. Kimball Research Institute of the New York Blood Center, New York, NY. |
| 2:30 | 396 | PRIMARY STRUCTURE AND SUBCELLULAR LOCALIZATION OF AN <u>ONCHOCERCA VOLVULUS</u> LOW MOLECULAR WEIGHT ANTIGEN. E. Lobos*, N. Weiss and T.B. Nutman. National Institutes of Health, Bethesda, MD; and Tropical Institute, Basel, SWITZERLAND. |
| 2:45 | 397 | IDENTIFICATION OF A FILARIAL ANTIGEN IN SERA FROM PATIENTS WITH ONCHOCERCIASIS. R. Chandrashekar*, A.F. Ogunrinade, O.O. Kale, and G.J. Weil. Washington University, St. Louis, MO and University of Ibadan, Ibadan, NIGERIA. |
| 3:00 | COFFEE BREAK | |
| 3:30 | 398 | SPECIFIC AND CROSS-REACTING ANTIBODIES IN HUMAN RESPONSES TO <u>ONCHOCERCA VOLVULUS</u> AND <u>DRACUNCULUS MEDINENSIS</u> . M.M. Kliks*, R.M.E. Parkhouse, T. Garate and Z. Cabrerra. University of Hawaii, Honolulu, HI; National Institute of Medical Research, UK. |
| 3:45 | 399 | IVERMECTIN UPTAKE AND DISTRIBUTION IN THE PLASMA AND TISSUES OF SUDANESE AND MEXICAN PATIENTS INFECTED WITH <u>ONCHOCERCA VOLVULUS</u> . M.H. El Kassaby*, C.K. Marschke, T.G. Geary, A. Rivas A., and J.F. Williams. Michigan State University, East Lansing, MI; Centro de Investigaciones Ecologicas del Sureste, Chiapas, MEXICO; and the Upjohn Company, Kalamazoo, MI. |

WEDNESDAY AFTERNOON, DECEMBER 13

SCIENTIFIC SESSION R: FILARIASIS - ONCHOCERCIASIS AND IVERMECTIN (Continued)

- 4:00 400 MASS TREATMENT OF ONCHOCERCA VOLVULUS INFECTION WITH IVERMECTIN. B.M. Greene, M.C. Pacque, B. Munoz, Z. Dukuly, A. Nara, C. Elmets, A.T. White, and H.R. Taylor. Johns Hopkins University, Baltimore, MD; Case Western Reserve University, Cleveland, OH; and The University of Alabama at Birmingham, Birmingham, AL.
- 4:15 401 EFFECTS OF MASS TREATMENT OF A HUMAN POPULATION WITH IVERMECTIN ON TRANSMISSION OF ONCHOCERCIASIS IN LIBERIA WEST AFRICA. M. Trpis, J.E. Childs, D.J. Fryauff, B.M. Greene, P.N. Williams, B.E. Munoz, M. Pacque, and H.R. Taylor. The Johns Hopkins University, Baltimore, MD; The University of Alabama at Birmingham, AL; Medical Center of the IAC, LIBERIA.
- 4:30 402 TRANSMISSION BLOCKING ACTIVITY OF IVERMECTIN IN BRUGIAN FILARIAL INFECTIONS. U.R. Rao*, B.H. Kwa, J.K. Nayar, and A.C. Vickery. University of South Florida, Tampa, FL; and University of Florida, Vero Beach, FL.
- 4:45 403 DOUBLE BLIND COMPARATIVE STUDY OF IVERMECTIN AND DIETHYLCARBAMAZINE (DEC) IN BANCROFTIAN FILARIASIS: ACTIVITY AGAINST THE ADULT STAGE OF THE PARASITE. F. Richards*, D. McNeeley, R. Bryan, M. Eberhard, M. McNeeley, P. Lammie, Y. Bernard, and H. Spencer. Centers for Disease Control, Atlanta, GA; and Hospital Ste. Croix, Leogane, HAITI.

SCIENTIFIC SESSION S: MALARIA - PRE-ERYTHROCYTIC STAGES

1:30 PM - 5:15 PM

Coral IV

Chairpersons: S. Chang and D. Lanar

Time Abstract

- 1:30 404 CIRCUMSPOROZOITE PROTEIN HETEROGENEITY IN THE HUMAN MALARIA PARASITE PLASMODIUM VIVAX. R. Rosenberg*, R. Wirtz, D. Lanar, J. Sattabongkot, T. Hall, A. Waters, and C. Prasittisuk. AFRIMS, Bangkok, THAILAND; Walter Reed Army Institute of Research, Washington, DC; National Institute of Health, Bethesda, MD; and Ministry of Public Health, Bangkok, THAILAND.
- 1:45 405 SEQUENCE ANALYSIS OF NATURALLY OCCURRING VARIANT CS GENE REPEATS OF PLASMODIUM VIVAX. D.E. Lanar*, R. Wirtz, and R. Rosenberg. Walter Reed Army Institute of Research, Washington, DC and Armed Forces Research Institute of Medical Sciences, Bangkok, THAILAND.
- 2:00 406 FURTHER CHARACTERIZATION OF TARGET ANTIGENS OF MALARIA TRANSMISSION-BLOCKING IMMUNITY. B. Wikel*, Z. Hong and N. Kumar. John Hopkins University, Baltimore, MD.

WEDNESDAY AFTERNOON, DECEMBER 13

SCIENTIFIC SESSION S: MALARIA - PRE-ERYTHROCYTIC STAGES (Continued)

- 2:15 407 PREFERENTIAL INDUCTION OF ANTIBODY SPECIFIC FOR THE REPEAT OR FLANKING REGIONS OF THE CS PROTEIN OF P. FALCIPARUM. J.S. Sundy*, M.M. Elloso, M. Gross, M.F. Good, and W.P. Weidanz. Hahnemann University, Philadelphia, PA; Smith Kline and French Laboratories, King of Prussia, PA; and Queensland Institute for Medical Research, Brisbane, AUSTRALIA.
- 2:30 408 CD4+ T CELL CLONES FROM A P. FALCIPARUM SPOROZOITE IMMUNIZED VOLUNTEER RECOGNIZE A T CELL EPITOPE WITHIN THE REPEAT REGION OF THE CS PROTEIN. E.H. Nardin*, D. Herrington, M. Levine, D. Stuber, P. Barr, R. Altszuler, P. Clavijo, and R.S. Nussenzweig. New York University School of Medicine, New York, NY; University of Maryland, Baltimore, MD; Hoffman-La Roche, Basel, SWITZERLAND; and Chiron Corporation, Emeryville, CA.
- 2:45 409 A PROCESSING DEPENDENT EPITOPE IS EXPRESSED IN THE CS PROTEIN OF SEVERAL PLASMODIAL SPECIES. M. Tsuji*, R.S. Nussenzweig and F. Zavala. New York University School of Medicine, New York, NY.
- 3:00 COFFEE BREAK
- 3:30 410 EFFECT OF INGESTED ANTI-SPOROZOITE ANTIBODIES ON SUBSEQUENT SPOROZOITE TRANSMISSION BY MOSQUITOES. J.A. Vaughan, L.F. Scheller, R.A. Wirtz, M.R. Hollingdale, and A.F. Azad. University of Maryland School of Medicine, Baltimore, MD, Walter Reed Army Institute of Research, Washington, DC, Biomedical Research Institute, Rockville, MD.
- 3:45 411 HUMAN HEPATOCYTE MEMBRANE PROTEINS 20 KD AND 55 KD SPECIFICALLY BIND TO PLASMODIUM FALCIPARUM SPOROZOITES. J. van Pelt, J. Kleuskens, M. Schepens, J-P Verhave, S.H. Yap, and M.R. Hollingdale. University of Nijmegen Medical School, THE NETHERLANDS, and Biomedical Research Institute, Rockville, MD.
- 4:00 412 RIBOSOMAL SWITCH AFTER INVASION OF PLASMODIUM BERGHEI SPOROZOITES IN CULTURED HEPATIC CELLS. J. Zhu, A. Appiah, T.F. McCutchan, A.P. Waters, A. Lal, and M.R. Hollingdale. Biomedical Research Institute, Rockville, MD; National Institute of Allergy and Infectious Diseases, Bethesda, MD.
- 4:15 413 PRODUCTION, PURIFICATION AND CHARACTERIZATION OF A HUMAN MONOCLONAL IgM ANTIBODY TO THE REPEAT REGION OF PLASMODIUM FALCIPARUM CS PROTEIN. S. Futrovsky, M.R. Hollingdale, J. Sadoff, L. Iccayan, A. Appiah, D. Monheit, W.R. Ballou, J. Chulay, and D.M. Gordon. Walter Reed Army Institute of Research, Washington, DC; Biomedical Research Institute, Rockville, MD; Univax Corporation, Rockville, MD.

WEDNESDAY AFTERNOON, DECEMBER 13

SCIENTIFIC SESSION S: MALARIA - PRE-ERYTHROCYTIC STAGES (Continued)

- 4:30 414 IMMUNOLOGICAL CHARACTERIZATION OF A RECOMBINANT PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN DEVOID OF REPEATS. M. Gross, D.M. Gordon, C. Silverman, G.F. Wasserman, M.C. Seguin, D. Sylvester, and M.R. Hollingdale. Smith Kline and French Laboratories, Swedeland, PA; Walter Reed Army Institute of Research, Washington, DC; Biomedical Research Institute, Rockville, MD.
- 4:45 415 PLASMODIUM FALCIPARUM SPOROZOITE ANTIGEN ASSOCIATED WITH PROTECTION OF MICE TO P. BERGHEI SPOROZOITE INFECTION. B. Sina, W. Weiss, V. Harrod, V.E. do Rosario, S. Aley, D. Hayes, J.F.G.M. Meis, and M.R. Hollingdale. Biomedical Research Institute, Rockville, MD; Naval Medical Research Institute, Bethesda, MD; University of Nijmegen Medical School, THE NETHERLANDS.
- 5:00 416 MHC-UNRESTRICTED ANTIBODY FORMATION TO REPETITIVE MALARIA ANTIGENS. L. Schofield and P. Uadia. New York University School of Medicine, New York, NY.

CLINICAL TROPICAL MEDICINE GROUP MEETING

1:30 PM - 5:30 PM

South Pacific I - II

Chairpersons: F.J. Bia and M. Wittner

Time Abstract

- 1:30 417 NEW TRENDS IN THE DIAGNOSIS AND TREATMENT OF FILARIASIS. E.A. Ottesen. Laboratory for Parasitic Diseases, National Institutes of Health, Bethesda, MD.
- 2:30 QUESTIONS AND ANSWERS
- 2:40 418 UNKNOWN CASE PRESENTATIONS IN CLINICAL TROPICAL MEDICINE. F.J. Bia, J.S. Keystone, and M. Wittner. Yale University School of Medicine, New Haven, CT; Toronto General Hospital, Toronto, CANADA; and Albert Einstein College of Medicine, New York, NY.
- 3:00 COFFEE BREAK
- 3:30 419 MALARIA UPDATE 1989: MEFLOROQUINE SAFETY AND EFFICACY. C.C. Campbell and H.O. Lobel. Malaria Branch, Centers for Disease Control, Atlanta, GA.
- 4:30 420 BUSINESS MEETING. E. Jong. University of Washington, Seattle, WA.

WEDNESDAY AFTERNOON, DECEMBER 13

30TH ANNUAL OPEN MEETING OF THE AMERICAN COMMITTEE
ON ARTHROPOD-BORNE VIRUSES

BUSINESS MEETING

1:30 PM - 2:30 PM

South Pacific III - IV

Chairperson: J.W. LeDuc

2:15 421 Nat Young Award

SCIENTIFIC SESSION: APPLICATION OF MOLECULAR TECHNIQUES TO
ARBOVIRUS EPIDEMIOLOGY

2:30 PM - 5:30 PM

South Pacific III - IV

Chairperson: R.E. Shope

Time Abstract

2:30	422	INTRODUCTION. R.E. Shope. Yale Arbovirus Research Unit, Yale University, New Haven, CT.
2:35	423	RNA VIRUS EVOLUTION AND EPIDEMIOLOGY DETERMINED BY LIMITED GENOMIC SEQUENCING. R. Rico-Hesse. Yale Arbovirus Research Unit, Yale University, New Haven, CT.
3:05		COFFEE BREAK
3:35	424	ENVIRONMENTAL RELEASE OF ORGANISMS WITH MOLECULAR MARKERS. D.H.L. Bishop, NERC Institute of Virology, Oxford, UK.
4:05	425	NUCLEOTIDE SEQUENCE OF THE 26S RNAs OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUSES P676, 3880, AND EVERGLADES. R.M. Kinney*, J.M. Sneider, K.R. Tsuchiya, and D.W. Trent. Centers for Disease Control, Division of Vector-Borne Viral Diseases, Fort Collins, CO.
4:35	426	CHARACTERISTICS OF A VARIANT STRAIN OF SIMIAN HEMORRHAGIC FEVER VIRUS ASSOCIATED WITH THE 1989 EPIZOOTIC OF NEW MEXICO. P.B. Jahrling, P.M. Zack, R.O. Cannon, and D.M. Renquist. USAMRIID, Fort Detrick, Frederick, MD; Centers for Disease Control, Atlanta, GA and Primate Research Institute, Alamogordo, NM.
5:00	427	EPIDEMIC REPORTS.

WEDNESDAY AFTERNOON - DECEMBER 13

SCIENTIFIC SESSION T: EPIDEMIOLOGY

1:30 PM - 4:30 PM

Nautilus II - III

Chairpersons: H.C. Spencer and S.H. Waterman

Time Abstract

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| 1:30 | 428 | EVALUATION OF CLINICAL CASE DEFINITIONS FOR MALARIA IN URBAN, CENTRAL AFRICA. N. Shaffer*, K. Hedberg, F. Davachi, L. Bongo, A. Vernon, B. Miaka, P. Nguyen-Dinh, and J.G. Breman. Centers for Disease Control, Atlanta, GA; and Mama Yemo Hospital and PEV/CCCD, Kinshasa, ZAIRE. |
| 1:45 | 429 | EPIDEMIOLOGICAL MODELS FOR SCHISTOSOMIASIS: A VARIABLE RATE APPROACH. A.D. Long. Agency for International Development, Washington, DC. |
| 2:00 | 430 | INJECTION AS A RISK FACTOR FOR PARALYTIC POLIOMYELITIS. R.W. Sutter*, P.A. Patriarca, S.L. Cochi, and S. Brogan. Centers for Disease Control, Atlanta, GA, and Ministry of Health, Muscat, OMAN. |
| 2:15 | 431 | THE INFLUENCE OF HOUSEHOLD CHARACTERISTICS ON DIARRHEA OCCURENCE IN RURAL EGYPT. M. Habib*, C.E. Wright, M. El Alamy, and H.L. DuPont. Epidemiology Study Center-Bilbeis, Egypt; The University of Texas Health Science Center, Houston, TX; Centers for Disease Control, Atlanta, GA. |
| 2:30 | 432 | ETIOLOGY OF UPPER RESPIRATORY DISEASE AMONG CHILDREN IN CAIRO, EGYPT. R.L. Haberberger*, N. Osman, R. Elyazeed, and D.M. Watts. U.S. Naval Medical Research Unit No. 3, Cairo, EGYPT; Al-Azhar Women's University, Cairo, EGYPT; and Ministry of Health, Assiut, EGYPT. |
| 2:45 | 433 | CLINICAL-EPIDEMIOLOGIC STUDY OF CHAGAS' DISEASE IN BOLIVIA. M. Pless*, D. Juranek, P. Kozarsky, F. Steurer, G. Tapia, and H. Bernudez. Emory University School of Medicine, Atlanta, GA; Center for Disease Control, Atlanta, GA; San Simon University, Cochabamba, BOLIVIA. |
| 3:00 | | COFFEE BREAK |
| 3:30 | 434 | TRANSMISSION AND EPIDEMIOLOGY OF MALARIA IN MADAGASCAR. J.P. Lepers, D. Fontenille, P. Deloron*, M.D. Andriamangatiana-Rason, and P. Coulanges. Institut Pasteur de Madagascar, Antananarivos, MADAGASCAR, and INSERM Unite 13, Paris, FRANCE. |

WEDNESDAY AFTERNOON, DECEMBER 13

SCIENTIFIC SESSION T: EPIDEMIOLOGY (Continued)

- 3:45 435 HYPERENDEMIC HUMAN AND BOVINE FASCIOLIASIS IN A RURAL BOLIVIAN COMMUNITY. R.T. Bryan*, J. Bjorland, C. Espindola, M. Lagrava, W. Agreda, D. Everaert, M. Vilca, A. Quiton, M. Soler, and G. Hillyer. Centers for Disease Control, Atlanta, GA; Proyecto Danchurchaid, PIL, Cordepaz, La Paz, BOLIVIA; Ministry of Health, BOLIVIA; Foster Parents Plan International, La Paz, BOLIVIA; University of Puerto Rico, San Juan, PUERTO RICO.
- 4:00 436 AN EPIDEMIOLOGICAL STUDY OF THE PREVALENCE AND SYMPTOMS OF ENTEROBIUS VERMICULARIS INFECTIONS IN A LIMA, PERU SHANTY TOWN. R.H. Gilman, G.S. Marquis and E. Miranda. The Johns Hopkins University, Baltimore, MD; Universidad Peruana Cayetano Heredia; and Instituto de Investigacin Nutricional.??
- 4:15 437 CHICKENPOX IN THE U.S. ARMY: A DEVELOPING EPIDEMIC OF MAJOR PROPORTIONS. J. Sanchez*, R. Miller, J. Longfield, and E. Takafuji. Walter Reed Army Institute of Research, Washington DC, Brook Army Medical Center, San Antonio, TX; Office of the Army Surgeon General, Falls Church, VA.

THURSDAY MORNING - DECEMBER 14

SYMPOSIUM: INTERNATIONAL TRAVEL MEDICINE

8:00 AM - 10:30 AM

Coral III - IV

Chairpersons: H.O. Lobel and R. Steffen

Time Abstract

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| 8:00 | 438 | MALARIA AND MALARIA PREVENTION AMONG U.S. TRAVELERS.
H.O. Lobel, Centers for Disease Control, Atlanta, GA. |
| 8:10 | 439 | MALARIA CHEMOPROPHYLAXIS: ADVANTAGES AND DISADVANTAGES.
J.S. Keystone. Toronto General Hospital, Toronto, CANADA. |
| 8:35 | 440 | PANEL DISCUSSION: MALARIA PREVENTION.
H.O. Lobel, Centers for Disease Control, Atlanta, GA.
J.S. Keystone, Toronto General Hospital, Toronto, CANADA.
S.L. Hoffmann, Malaria Program, Naval Medical Research
Institute, Bethesda, MD. |
| 9:00 | 441 | TRAVELERS' DIARRHEA. H.L. DuPont. University of Texas
Medical School, Houston, TX. |
| 9:25 | 442 | PANEL DISCUSSION: TRAVELERS' DIARRHEA.
H.L. DuPont, University of Texas Medical School, Houston,
TX.
R.L. Guerrant, University of Virginia Medical School,
Charlottesville, VA.
M.S. Wolfe, Traveler's Medical Service, Washington, DC. |
| 9:45 | 443 | THE EPIDEMIOLOGICAL BASIS FOR VACCINATION OF TRAVELERS.
R. Steffen, University of Zurich Medical School, Zurich,
SWITZERLAND. |
| 10:10 | 444 | PANEL DISCUSSION: VACCINATION STRATEGIES FOR TRAVELERS
IN 1990.
R. Steffen, University of Zurich Medical School, Zurich,
SWITZERLAND.
M. Barry, Yale University School of Medicine, New Haven, CT.
E. Jong, University of Washington Medical School, Seattle,
WA. |

SCIENTIFIC SESSION U: FILARIASIS - MOLECULAR BIOLOGY

8:00 AM - 10:30 AM

South Pacific I - II

Chairpersons: T.V. Rajan and S. Williams

Time Abstract

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| 8:00 | 445 | CLONING OF A HIGHLY REPEATED PROTEIN LOCATED IN THE GUT OF
FILARIAL PARASITES. L.A. McReynolds*, C. Poole, G. Grandea,
R. Maizles, M. Selkirk, and R. Jenkins. New England
Biolabs, Beverly, MA, Imperial College of Science and
Technology and London School of Hygiene and Tropical
Medicine, London, UK. |
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THURSDAY MORNING, DECEMBER 14

SCIENTIFIC SESSION U: FILARIASIS - MOLECULAR BIOLOGY (Continued)

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| 8:15 | 446 | IN SITU HYBRIDIZATION: A METHOD OF RELATING CLONED ANTIGENS TO THEIR SITE OF EXPRESSION WITHIN INDIVIDUAL FILARIAL WORMS AND LARVAE. F.B. Perler*, D. Abraham, M. Mulligan, J.B. Lok, and R. Tuan. New England Biolabs, Beverly, MA; Thomas Jefferson University and University of Pennsylvania, Philadelphia, PA. |
| 8:30 | 447 | MOLECULAR GENETICS OF FILARIAL MYOSINS. C. Werner* and T.V. Rajan. Albert Einstein College of Medicine, Bronx, NY, and University of Connecticut Health Center, Farmington, CT. |
| 8:45 | 448 | EXPRESSION OF <u>BRUGIA MALAYI</u> 70 KD Heat Shock Genes. N.M. Rothstein* and T.V. Rajan. University of Connecticut Health Center, Farmington, CT. |
| 9:00 | 449 | AN EXAMINATION OF THE PARAMETERS INVOLVED IN PURIFICATION OF PARASITE ANTIGEN FUSION PROTEINS. M.W. Southworth*, S.E. Roemer, and F.B. Perler. New England Biolabs, Beverly, MA. |
| 9:15 | 450 | ISOLATION AND CHARACTERIZATION OF REPETITIVE DNA CLONES OF <u>WUCHERERIA BANCROFTI</u> . N. Raghavan*, S.A. Williams, E.A. Ottesen and T.B. Nutman. National Institute of Allergy and Infectious Diseases, Bethesda, MD, and Smith College, Northampton, MA. |
| 9:30 | 451 | ISOLATION AND PARTIAL CHARACTERIZATION OF REPETITIVE DNA SEQUENCES FROM <u>LOA LOA</u> . A.D. Klion and T.B. Nutman. National Institutes of Health, Bethesda, MD. |
| 9:45 | 452 | ISOLATION OF A MEMBER OF REPEATED SEQUENCE FAMILY SPECIFIC FOR SAVANNAH FORM <u>O. VOLVULUS</u> . K.D. Erttmann, P. Zimmerman, B.M. Greene, and T.R. Unnasch*. University of Alabama at Birmingham, Birmingham, AL. |
| 10:00 | 453 | PCR AMPLIFICATION AND NON-RADIOACTIVE DNA PROBES FOR THE SPECIES SPECIFIC DETECTION OF <u>BRUGIA</u> AND <u>WUCHERERIA</u> IN HUMAN BLOOD SAMPLES. S.A. Williams*, J.G. Glover, S.J. Szabo, L.A. McReynolds, D. Landry, and F. Partono. Smith College, Northampton, MA; New England Biolabs, Beverly, MA and University of Indonesia, Jakarta, INDONESIA. |
| 10:15 | 454 | FIELD TESTING OF A MONOCLONAL PROBE SPECIFIC FOR INFECTIVE LARVAE OF HUMAN BRUGIAN PARASITES. C.K.S. Carlow, A. Suwita, Z. Gahang, Purnomo, F. Partono, and M. Philipp. New England Biolabs, Beverly, MA; University of Indonesia, and Ministry of Health, Jakarta, INDONESIA. |

THURSDAY MORNING - DECEMBER 14

SCIENTIFIC SESSION V: VIRUS VACCINES

8:00 AM - 10:30 AM

South Pacific III - IV

Chairpersons: P.K. Russell and A.L. Schmaljohn

<u>Time</u>	<u>Abstract</u>	
8:00	455	HUMORAL IMMUNITY TO VACCINIA VIRUS. A.L. Schmaljohn*, J.W. Hooper and S.A. Harrison. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
8:15	456	PROTECTION OF NONHUMAN PRIMATES AGAINST VENEZUELAN ENCEPHALITIS AFFORDED BY A RECOMBINANT VACCINIA VACCINE. T.P. Monath*, C.B. Cropp, W. Short, R.M. Kinney, and D.W. Trent. Centers for Disease Control, Fort Collins, CO, and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
8:30	457	RECOMBINANT VACCINIA-VENEZUELAN EQUINE ENCEPHALITIS VIRUS (VEE) PROTECTS HORSES FROM PERIPHERAL VEE VIRUS CHALLENGE. R.A. Bowen, C.B. Cropp, W. Short, R.M. Kinney, T.P. Monath, and D.W. Trent*. Colorado State University, and Centers for Disease Control, Fort Collins, CO.
8:45	458	EXPRESSION OF HANTAAVIRUS ANTIGENS FOR RECOMBINANT VACCINE DEVELOPMENT. C.S. Schmaljohn, A.L. Schmaljohn, Y.K. Chu and J.M. Dalrymple. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
9:00	459	BLUETONGUE VIRUS SUBUNIT VACCINES. P. Roy*. NERC Institute of Virology and Environmental Microbiology, Oxford, UK and University of Alabama at Birmingham, Birmingham, AL.
9:15	460	SAFETY AND EFFICACY OF A MUTAGEN-ATTENUATED RIFT VALLEY FEVER VACCINE IN PREGNANT BOVIDS. J.C. Morrill*, C.A. Mebus, R.G. Breeze, and C.J. Peters. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD and The Plum Island Animal Disease Center, Greenport, NY.
9:30	461	ORAL IMMUNIZATION USING LIVE ATTENUATED RIFT VALLEY FEVER VIRUS STRAINS. M.L.M. Pitt* and A.O. Anderson. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
9:45	462	PROTECTIVE EFFICACY IN RATS OF A FORMALIN-INACTIVATED RIFT VALLEY FEVER VIRUS (RVFV) VACCINE AGAINST AEROSOL CHALLENGE. G.W. Anderson, Jr.*, J.O. Lee, A.O. Anderson, N. Powell, and G. Meadors. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

THURSDAY MORNING, DECEMBER 14

SCIENTIFIC SESSION V: VIRUS VACCINES (Continued)

- 10:00 463 T-CELL DETERMINANTS ON LASSA VIRUS GLYCOPROTEIN (GP-C).
V.J. La Posta* and G.A. Cole. University of Maryland at
Baltimore, Baltimore, MD.
- 10:15 464 NEUTRALIZING ANTIBODY FOLLOWING FORMALIN INACTIVATED
HEPATITIS A VACCINE: PERSISTENCE OF ANTIBODY AND
NEUTRALIZATION OF ISOLATES FROM THREE CONTINENTS. L.N.
Binn*, M. Sjogren, R.H. Marchwicki, C. Hoke, and W.
Bancroft. Walter Reed Army Institute of Research,
Washington, DC.

SCIENTIFIC SESSION W: KINETOPLASTIDIA - BIOCHEMISTRY,
MOLECULAR BIOLOGY AND CHEMOTHERAPY

8:00 AM - 10:30 AM

Nautilus II - III

Chairpersons: K.P. Chang and A. Clarkson

Time Abstract

- 8:00 465 CHARACTERIZATION OF AN RNA VIRUS FROM THE PARASITE
LEISHMANIA. B. Widmer, A.M. Comeau, D.B. Furlong, D.P.
Wirth and J.L. Patterson. Harvard Medical School and
Harvard School of Public Health, Boston, MA.
- 8:15 466 MONOCLONAL ANTIBODIES AGAINST T. CRUZI NEURAMINIDASE REVEAL
ENZYME POLYMORPHISM AMONG DIFFERENT STRAINS, RECOGNIZE A
SUBSET OF PARASITES AND ENHANCE INFECTION IN VITRO. R.P.
Prioli* and M.E.A. Pereira. New England Medical Center
Hospitals, Boston, MA.
- 8:30 467 INHIBITION OF ANTIMONY-RESISTANT LEISHMANIA WITH A BIS
(BENZYL) POLYAMINE ANALOG. R.J. Baumann*, A.J. Bitonti,
P.P. McCann, W.L. Hanson, and A. Sjoerdsma. Merrel Dow
Research Institute, Cincinnati, OH and the University of
Georgia, Athens, GA.
- 8:45 468 EFFECTS OF ETHER ANALOGUES OF LYSOPHOSPHOLIPIDS ON
LEISHMANIA. B.Z. Ngwenya* and J. Wiltschko-Scott. Himmelman
University School of Medicine, Philadelphia, PA.
- 9:00 469 METABOLIC PREADAPTATION OF METACYCLIC AFRICAN TRYPANOSOMES
FOR THE MAMMALIAN BLOODSTREAM. J. Kiara, W.R. Fildes, and
E.J. Bienen. University of Nairobi and International
Laboratory for Research on Animal Diseases, Nairobi, KENYA.
- 9:15 470 MITOCHONDRIAL PROTON MOTIVE FORCE IN BLOODSTREAM AFRICAN
TRYPANOSOMES DEMONSTRATED BY RHODAMINE 123. M. Sario*, E.J.
Bienen, G. Pollakis, R.W. Grady, and A.B. Clarkson, Jr. New
York University Medical Center, New York, NY; ICBAD,
Nairobi, KENYA; and Cornell University Medical Center, New
York, NY.

THURSDAY MORNING, DECEMBER 14

SCIENTIFIC SESSION W: KINETOPLASTIDIA (Continued)

- 9:30 471 INVOLVEMENT OF HUMAN TRANSFERRIN IN THE TRANSFER OF IRON TO TRYPANOSOMA CRUZI. M.F. Lima* and F. Villalta. Meharry Medical College, Nashville, TN.
- 9:45 472 IDENTIFICATION OF A GENE WHICH IS DIFFERENTIALLY EXPRESSED DURING DEVELOPMENT OF TRYPANOSOMA RHODESIENSE FROM BLOODSTREAM TO PROCYCLIC TRYPOMASTIGOTES. L.E. Wirtz, D.A. Sylvester, and G.C. Hill. Meharry Medical College, Nashville, TN.
- 10:00 473 DNA METHYLATION PATTERNS OF TRYPANOSOMA BRUCEI BRUCEI DURING DFMO INDUCED TRANSFORMATION IN VIVO. B.F. Giffin* and S.J. Wunderle. University of Dayton, Dayton, OH.
- 10:15 474 DIFFERENTIAL SENSITIVITY OF TRYPANOSOMA B. RHODESIENSE CLINICAL ISOLATES TO DIFLUOROMETHYLORNITHINE AND ARSENICALS. C.J. Bacchi*, H. Nathan, N. Yarlett, P. Sayer, A. Njogu, P.P. McCann, A.J. Bitonti, and A.B. Clarkson, Jr. Pace University, New York, NY; Kenya Trypanosomiasis Research Institute (KETRI), Muguga, KENYA; Merrell Dow Research Institute, Cincinnati, OH; New York University Medical Center, New York, NY.

SCIENTIFIC SESSION X: SCHISTOSOMIASIS - ANTIGENS AND IMMUNOGENS

8:30 AM - 10:30 AM

Sea Pearl III - VI

Chairpersons: S.L. James and D.G. Colley

Time Abstract

- 8:30 475 IDENTIFICATION AND CHARACTERIZATION OF GLYCOSYL-PHOSPHATIDYLINOSITOL - LINKED SCHISTOSOMA MANSONI ADULT WORM IMMUNOGENS. S.Y. Sauma* and N. Strand. Johns Hopkins University School of Medicine, Baltimore, MD.
- 8:45 476 EVIDENCE OF CROSS-REACTIVE, SHARED IDIOTYPES ON ANTI-SEA ANTIBODIES FROM HUMANS AND MICE WITH SCHISTOSOMIASIS. M.A. Montesano*, G.L. Freeman, G. Gazzinelli and D.G. Colley. Universidad Federale Juiz de Fora, Juiz de Fora, MG, BRAZIL and VA Medical Center and Vanderbilt University School of Medicine, Nashville, TN.
- 9:00 477 CHARACTERIZATION OF TRIOSE PHOSPHATE ISOMERASE cDNA AND GENOMIC CLONES FROM SCHISTOSOMA MANSONI. M.G. Reis*, A. Gross, D. Harn and C. Shoemaker. Harvard School of Public Health, Boston, MA.
- 9:15 478 PEPTIDE FRAGMENTS OF RECOMBINANT SCHISTOSOME TRIOSE PHOSPHATE ISOMERASE RECOGNIZED BY ANTI-23 KD ANTIBODIES. D.A. Harn*, and W. Gu. Harvard School of Public Health, Boston, MA.

THURSDAY MORNING, DECEMBER 14

SCIENTIFIC SESSION X: SCHISTOSOMIASIS - ANTIGENS AND IMMUNOGENS (Continued)

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| 9:30 | 479 | SCHISTOSOMA MANSONI EGF RECEPTOR HOMOLOGUE: CLONING AND CHARACTERIZATION. C.B. Shoemaker*, M.R. Reis, A. Landa, and L. Stein. Harvard School of Public Health, Boston, MA. |
| 9:45 | 480 | AUGMENTED HUMAN ANTIBODY RESPONSE TO SMW68, A CANDIDATE S. MANSONI VACCINE IS ASSOCIATED WITH PROTECTION AGAINST REINFECTION FOLLOWING THERAPY. C.H. King*, M. Amer, A. El Hawey, and A.A.F. Mahmoud. Al-Azhar University, Cairo, EGYPT, and Case Western Reserve University, Cleveland, OH. |
| 10:00 | 481 | IDENTIFICATION OF A DONINANT EPITOPE ON THE CYSTEINE PROTEINASE FROM S. MANSONI ADULT WORMS. F. Monroy, M.H. Dresden, and C.L. Chappell*. Verna and Marrs McLean. Baylor College of Medicine, Houston, TX. |
| 10:15 | 482 | SCHISTOSOMA MANSONI: THE ROLE OF CALCIUM IN THE REGULATION OF CERCARIAL PROTEINASE RELEASE. A.C. Fusco* and B. Salafsky. University of Illinois College of Medicine at Rockford, Rockford, IL. |

ANNUAL FRED SOPER LECTURE

11:00 AM - 12:00 NOON

Coral III - IV

11:00 Introduction by F.A. Neva

Time Abstract

11.05	483	IN SEARCH OF A NATIONAL AGENDA FOR INTERNATIONAL HEALTH PROBLEMS. W.H. Foege. Carter Center, Atlanta, GA.
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THURSDAY AFTERNOON - DECEMBER 14

SYMPOSIUM: HANSEN'S DISEASE: ADVANCES IN CLINICAL AND
EXPERIMENTAL RESEARCH*

1:30 PM - 5:00 PM

Coral III

Chairpersons: S.K. Nordeen and D.M. Scollard

Time Abstract

1:30		INTRODUCTION
1:40	484	GENETIC RESISTANCE TO INTRACELLULAR INFECTION. E. Skamene. Center for the Study of Host Resistance, McGill University, Montreal, CANADA.
2:10	485	GENETIC DETERMINANTS OF SPECIFIC IMMUNITY TO M. LEPRAE. R.R.P. de Vries. University Hospital, Leiden, THE NETHERLANDS.
2:40	486	INSIDE THE SKIN: THE LOCAL IMMUNE AND INFLAMMATORY MILIEU IN LEPROSY. D.M. Scollard. John A. Burns School of Medicine, University of Hawaii, Honolulu, HI.
3:00		COFFEE BREAK
3:15	487	LEPROSY IN NON-HUMAN PRIMATES -- NATURALLY ACQUIRED AND EXPERIMENTAL INFECTIONS. W.M. Meyers, President, International Leprosy Association; Mycobacteriology Section, Armed Forces Institute of Pathology, Washington, DC.
3:45	488	EPIDEMIOLOGY OF LEPROSY: THE CURRENT EPIDEMIC IN MICRONESIA. R. Worth and M. O'Leary. Hawaii State Health, Honolulu, HI; and Pohnpei, EASTERN CAROLINE ISLANDS.
4:05	489	SEROLOGY OF LEPROSY. J. Douglas. University of Hawaii, Honolulu, HI.
4:25	490	MOLECULAR IMMUNOLOGY OF NEW GENERATION VACCINES FOR HANSEN'S DISEASE. J.D. Watson. University of Auckland, NEW ZEALAND.
4:50		DISCUSSION AND CONCLUDING COMMENTS.

* - Co-sponsored by the John A. Burns School of Medicine, University of Hawaii and the Governor's Coordinating Committee for the Father Damien Centennial Celebration.

THURSDAY AFTERNOON - DECEMBER 14

SYMPOSIUM: MALARIA IN PREGNANCY*

1:30 PM - 5:30 PM

Coral IV

Chairpersons: M. Aikawa and R.S. Desowitz

<u>Time</u>	<u>Abstract</u>	
1:30	491	INTRODUCTION. R.S. Desowitz. University of Hawaii, Honolulu, HI.
1:35	492	EPIDEMIOLOGY OF MALARIA IN PREGNANCY IN AFRICA. R. Steketee. Centers for Disease Control, Atlanta, GA.
2:00	493	EPIDEMIOLOGY OF MALARIA IN PREGNANCY IN SOUTHEAST ASIA AND THE PACIFIC. G. Buchbinder. University of Hawaii, Honolulu, HI.
2:25	494	PLACENTAL PATHOLOGY IN HUMAN MALARIA. M. Aikawa. Case Western Reserve University, Cleveland, OH.
2:50		COFFEE BREAK
3:00	495	ANIMAL MODELS OF PREGNANCY-RELATED ENHANCED MALARIA. R. Desowitz. University of Hawaii, Honolulu, HI.
3:25	496	THE IMMUNE RESPONSE IN PREGNANCY-RELATED ENHANCED MALARIA. W. Eling. Catholic University, School of Medicine, Nijmegen, THE NETHERLANDS.
3:50	497	MATERNAL TRANSFER OF PROTECTIVE ANTIBODIES IN MALARIA. M. Hollingdale. Biomedical Research Institute, Rockville, MD.
4:15	498	TREATMENT AND PREVENTION OF MALARIA IN PREGNANCY. J. Breman. Centers for Disease Control, Atlanta, GA.
4:40	499	MATERNAL AND FETAL IMMUNE PHENOMENA ASSOCIATED WITH HUMAN PREGNANCY. A. Beer. The Chicago Medical School, Chicago, IL.
5:05		GENERAL DISCUSSION.
5:25	500	CLOSING REMARKS. M. Aikawa. Case Western Reserve University, Cleveland, OH.

* - This Symposium is supported by the Agency for International Development Malaria Immunity and Vaccine Research Program.

THURSDAY AFTERNOON - DECEMBER 14

SCIENTIFIC SESSION Y: RETROVIRAL INFECTIONS

1:30 PM - 2:45 PM

South Pacific I - II

Chairpersons: J. A. Fishman and S.H. Vermund

<u>Time</u>	<u>Abstract</u>
1:30	501 CLINICAL AND ENDOSCOPIC EVALUATION OF DIARRHEA AND WASTING IN KENYAN PATIENTS INFECTED WITH HIV. R.T. Bryan*, R.L. Owen, A. Cali, F.A. Okoth, J.B.O. Were, F. Sang, and H.C. Spencer. Centers for Diseases Control, Atlanta, GA; University of California, San Francisco, CA; Rutgers University, Newark, NJ; Kenya Medical Research Institute, Nairobi, KENYA.
1:45	502 TRENDS IN HIV SEROPOSITIVITY IN A PEDIATRIC EMERGENCY WARD PATIENT POPULATION, KINSHASA, ZAIRE. P. Nguyen-Dinh*, N. Shaffer, F. Davachi, K. Hedberg, L. Bongo, F. Behets, A.N. Vernon, Miaka mia Bilenge, and R.W. Ryder. Centers for Disease Control, Atlanta, GA;; Hospital Mama Yemo, Project SIDA, and PEV/CCCD, Kinshasa, ZAIRE.
2:00	503 SEROEPIDEMIOLOGICAL EVIDENCE OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I (HTLV-1) INFECTION AMONG THE HAGAHAI OF PAPUA NEW GUINEA. R. Yanagihara*, C.L. Jenkins, C. Mora, R.M. Garruto, and D.C. Gajdusek. National Institutes of Health, Bethesda, MD.
2:15	504 A RAPID IMMUNOBLOT ASSAY (WESTERN BLOT) TO DETECT SPECIFIC ANTIBODIES FOR HUMAN IMMUNODEFICIENCY VIRUS, <u>SCHISTOSOMA MANSONI</u> , AND <u>TAENIA SOLIUM</u> (CYSTICERCOSIS). J.A. Brand* and V.C.W. Tsang. Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.
2:30	505 INACTIVATION OF HIV IN BLOOD SAMPLES STORED AS HIGH SALT LYSATES. J.W. Zolg*, R.S. Lanciotti, and W.A. Meyer III. Biomedical Research Institute, Rockville, MD and Maryland Medical Laboratory Inc., Baltimore, MD.
2:45	COFFEE BREAK

THURSDAY AFTERNOON - DECEMBER 14

SYMPOSIUM: OPPORTUNISTIC INFECTIONS IN AIDS - NEW INSIGHTS*

3:00 PM - 4:40 PM

South Pacific I - II

Chairpersons: J.A. Fishman and S.H. Vermund

Time Abstract

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| 3:00 | 506 | INFECTIONS IN AIDS AND THE SEARCH FOR SPECIFIC <u>PNEUMOCYSTIS CARNI</u> ANTIGENS. J.A. Fishman. Massachusetts General Hospital and Harvard Medical School, Boston, MA. |
| 3:25 | 507 | <u>TOXOPLASMA GONDII</u> : MECHANISMS OF CELLULAR INVASION. K. Joiner. Yale University School of Medicine, New Haven, CT. |
| 3:50 | 508 | <u>MYCOBACTERIUM TUBERCULOSIS</u> : ASSESSING THE T-CELL RESPONSE. W.H. Boom. University of Cincinnati School of Medicine, Cincinnati, OH. |
| 4:15 | 509 | CANDIDA: CELL WALL GLYCOPROTEINS IN THE DETERMINATION OF VIRULENCE. J.E. Dormer. Tulane University Medical School, New Orleans, LA. |
| 4:30 | 510 | DISCUSSION AND CONCLUDING REMARKS. Moderator by J.A. Fishman. Massachusetts General Hospital and Harvard Medical School, Boston, MA. |

* - The symposium on Opportunistic Infections in AIDS is supported by the Burroughs-Wellcome Company.

SCIENTIFIC SESSION Z: MALARIA: CHEMOTHERAPY

1:30 PM - 5:00 PM

South Pacific III - IV

Chairpersons: W.K. Milhous and C.M. Wilson

Time Abstract

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| 1:30 | 511 | MOLECULAR GENETICS OF CHLOROQUINE RESISTANCE IN <u>PLASMODIUM FALCIPARUM</u> . T.E. Wellems*, L.J. Panton, D.J. Krogstad, I.Y. Gluzman, A. Walker-Jonah, V.E. do Rosario, R. Gwadz, and S.A. Dolan. National Institutes of Health, Bethesda, MD, and Washington University School of Medicine, St. Louis, MO. |
| 1:45 | 512 | TREATMENT OF CHLOROQUINE-RESISTANT MALARIA IN AOTUS MONKEYS WITH CHLOROQUINE AND A CALCIUM ANTAGONIST. S.K. Martin, H.L. Williams*, V.C. Okoye and D.J. Johnson. Walter Reed Army Institute of Research, Washington, DC. |

THURSDAY AFTERNOON - DECEMBER 14

SCIENTIFIC SESSION Z: MALARIA: CHEMOTHERAPY (Continued)

- 2:00 513 EFFECT OF WR-238605 ON THE SPOROGENIC DEVELOPMENT OF PLASMODIUM BERGHEI ANKA IN ANOPHELES STEPHENSI MOSQUITOES. R.E. Coleman* and R.A. Wirtz. Walter Reed Army Medical Institute of Research, Washington, DC.
- 2:15 514 MOLECULAR BASIS OF DIFFERENTIAL SENSITIVITY TO CYCLOGUANIL AND PYRIMETHAMINE IN FALCIPARUM MALARIA. D.S. Peterson*, W.K. Milhous, and T.E. Wellems. National Institute of Health, Bethesda, MD; Walter Reed Army Medical Institute of Research, Washington, DC.
- 2:30 515 MALARIA PREVENTION WITH MEFLOQUINE AMONG PEACE CORPS VOLUNTEERS. H.O. Lobel*, K.W. Bernard, and L.C. Patchen. Centers for Disease Control, Atlanta, GA; Medical Services, Peace Corps, Washington, DC.
- 2:45 516 P-GLYCOPROTEIN-LIKE MOLECULE IDENTIFIED IN PLASMODIUM FALCIPARUM. A.E. Serrano*, S.K. Volkman, C.M. Wilson, Z. Etizion, and D.F. Wirth. Harvard School of Public Health, Boston, MA and Rockefeller University, New York, NY.
- 3:00 COFFEE BREAK
- 3:30 517 DNA BINDING BY CHLOROQUINE: POSSIBLE MODE OF ACTION. F. Kwakye-Berko and S.R. Meshnik*. City University of New York Medical School, New York, NY.
- 3:45 518 IN VITRO REVERSAL OF MEFLOQUINE AND CHLOROQUINE RESISTANCE IN MULTI-DRUG RESISTANT PLASMODIUM FALCIPARUM ISOLATES FROM THAILAND. D.E. Kyle*, H.K. Webster, and W.K. Milhous. Walter Reed Army Medical Institute of Research, Washington, DC. and Armed Forces Institute for the Medical Sciences, Bangkok, THAILAND.
- 4:00 519 NEW WATER SOLUBLE ARTEMISININ (QINGHAOSU) DERIVATIVES AS ANTIMALARIAL AGENTS. A.J. Lin, M. Lee*, L.Q. Li, D.L. Klayman, W.K. Milhous and A.L. Ager. Walter Reed Army Medical Institute of Research, Washington, DC. and University of Miami, Miami, FL.
- 4:15 520 DOES THE INTRINSIC ACTIVITY OF PROGUNAIL CONTRIBUTE TO ITS ANTIMALARIAL EFFICACY? C.J. Canfield, K.B. Canfield, B.G. Schuster and W.K. Milhous*. Pharmaceutical Systems Inc., Gaithersburg, MD and Walter Reed Army Institute of Research, Washington, DC.

THURSDAY AFTERNOON, DECEMBER 14

SCIENTIFIC SESSION Z: MALARIA: CHEMOTHERAPY (Continued)

- 4:30 521 USE OF RIBOSOMAL RNA PROBES TO QUANTITATE MALARIA EXOERYTHROCYTIC DEVELOPMENT AND TO EVALUATE IN VITRO EFFECTS OF CHEMICAL ANTIMALARIALS. J. Li, J. Zhu, T.F. McCutchan, G. Long, A. Appiah, L. Graves, W.K. Milhous, and M.R. Hollingdale. Second Military Medical University, Shanghai, PEOPLE'S REPUBLIC OF CHINA; Biomedical Research Institute, Rockville, MD; Walter Reed Army Institute of Research, Washington, DC.; Naval Medical Research Institute, Bethesda, MD.
- 4:45 522 A DIAGNOSTIC TEST FOR CHLOROQUINE RESISTANCE. D.J. Krogstad*, I.Y. Gluzman, P.H. Schlesinger, A.U. Orjih, K. Nkangineme, and T.E. Wellems. Washington University School of Medicine, St. Louis, MO; University of Port Harcourt, Port Harcourt, NIGERIA; and National Institutes of Health, Bethesda, MD.

SYMPOSIUM: AMERICAN SOCIETY OF TROPICAL VETERINARY MEDICINE

SIGNIFICANCE OF NEW WORLD PRIMATES
IN TROPICAL AND RELATED DISEASES

1:30 PM - 5:00 PM

Nautilus II - III

Chairpersons: W.E. Collins and I. Kakoma

Time Abstract

- 1:15 523 INTERNATIONAL TECHNICAL CO-OPERATION ON THE CONSERVATION, REPRODUCTION, AND BIOMEDICAL USE OF TROPICAL PRIMATES. P. Arambulo, PAHO, Washington, DC.
- 1:55 524 CYTOGENETICS AND MOLECULAR CYTOGENETICS OF OWL MONKEYS. N. Ma. Harvard University, Cambridge, MA.
- 2:40 525 PATHOLOGY OF NON-HUMAN PRIMATES IN RELATION TO PROTOZOAL AND VIRAL DISEASES. R. Broderick. Centers for Disease Control, Atlanta, GA.
- 3:25 COFFEE BREAK
- 3:35 526 REPRODUCTIVE PHYSIOLOGY OF SQUIRREL MONKEYS. C. Abee. Mobile, Alabama.
- 4:05 527 MEDICAL MANAGEMENT OF SPONTANEOUS AND EXPERIMENTAL DISEASES. R.E. Weller. Battelle Laboratories, Richland, WA.
- 4:40 528 DISCUSSION: AN OVERVIEW OF THE IMPORTANCE OF NON-HUMAN PRIMATES IN MEDICAL RESEARCH. Introduced and moderated by: W.E. Collins, Centers for Disease Control, Atlanta, GA.

American Society of Tropical Medicine and Hygiene

December 11 - December 14, 1989

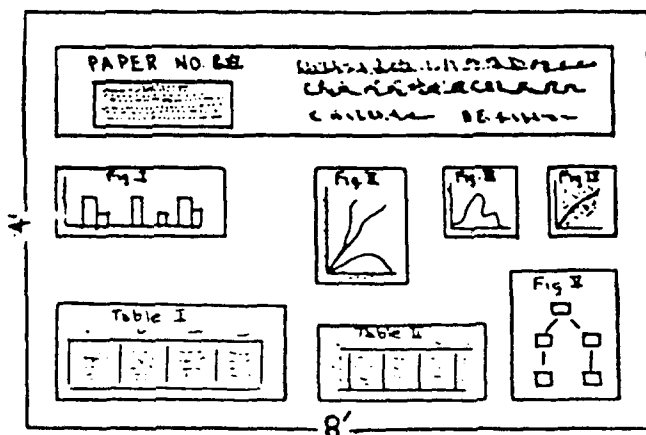
GUIDELINES FOR POSTER PRESENTATIONS

Abstracts scheduled for presentation in the poster session will be grouped by topic, numbered, and listed in the program.

The poster board surface area is 4' high and 8' wide. Prepare a label for the top of your poster space indicating the program number of your paper, its title and authors and their affiliations. The lettering for this section should be at least 2-3" high. A copy of your abstract should be posted in the upper left-hand corner of the poster board.

All illustrations should be made up beforehand. Bear in mind that your illustrations must be read by interested scientists from distances of 3' or more. Charts, drawings and illustrations might well be similar to those you would otherwise use in making slides; profitably cruder and more heavily drawn. Illustrations reproduced photographically should have a mat finish to avoid the glare produced by glossy prints. Simple use of color can add emphasis effectively. Do not mount illustrations on heavy board because it may be difficult to keep in position on the poster boards. Hand lettered material should contain appropriately heavy lettering at least 3/8" high. Typed material should be typed on a Bulletin (large type) typewriter, and photographically enlarged. Keep illustrative material simple. You might also find it useful to have on hand a tablet of suitable sketch paper (9" X 12") as well as one or two felt marking pens.

"Poster Assistants" will help you with any information or technical assistance you may need. A suggested arrangement of poster is illustrated below.



Provide a label containing the abstract number, title and name(s) of author(s) to identify your presentation easily.

Post a copy of the abstract.

Prepare and bring with you to the meeting all illustrations needed for your presentation - figures, tables, schemes, equations, etc.

Mount your labels and illustrations on the fiberboard with drawing pins (thumb-tacks). These items will be provided.

Please do not write or draw on the poster boards.

American Society of Tropical Medicine and Hygiene

December 11 - December 14, 1989

GUIDELINES FOR ORAL PRESENTATIONS

Time - Oral presentations in the regular scientific sessions may be up to 10 minutes in length, with an additional 5 minutes for discussion. The time allocated for oral presentations in symposia and workshops varies - as noted in the program.

Presentation - Please rehearse your presentation so it does not exceed the allotted time, and so that it is clearly coordinated with your slides. Giving the talk to several colleagues 1-2 weeks before the meeting is invariably helpful. It also allows enough time to revise slides that are unacceptable, and to make additional slides if they are needed.

Organization - In addition to the data being presented, each talk should explain why the studies were performed (what question was being asked) describe the methods, and provide a clear summary of the results.

Slide Composition - Slides must be legible from the back of the room. In general, 8-9 lines (including title, headings and data) are the maximum for one slide. Any slide introduced by "I know this is crowded, but,..." should be replaced.

Chairpersons - In fairness to the audience, chairpersons will ask speakers to explain the content of excessively crowded slides - rather than reviewing them line by line with the pointer.

To permit members to attend different sessions in the same morning afternoon, chairpersons have been asked to limit discussion to 5 minutes, and to recess for 15 minutes if a speaker does not show for his/her presentation.

NOTES

ABSTRACTS

THE 38TH ANNUAL MEETING
OF THE AMERICAN SOCIETY OF
TROPICAL MEDICINE AND HYGIENE

ASTMH OPENING PLENARY SESSION
(No abstracts available)

- 1 WELCOME AND DESCRIPTION OF THE PLENARY SESSION: L.H. Miller, President, ASTMH. National Institutes of Health, Bethesda, MD.
- 2 DESCRIPTION OF THE "GLOBAL HEALTH" SCENARIO AND THE OBJECTIVES OF THE EIWG. L.J. Legters, EIWG Chairperson. Uniformed Services University of the Health Sciences, Bethesda, MD.
- 3 IN-COUNTRY SITUATION AND MILITARY OVERVIEW. E.T. Takafuji, representing the Office of the Army Surgeon General, Falls Church, VA.
- 4 GLOBAL IMPACT. M.S. Wolfe, representing the Department of State, Washington, DC.
- 5 U.S. PUBLIC HEALTH CONCERNS. D.R. Hopkins, representing the National Institute for Allergy and Infectious Diseases and the Centers for Disease Control. Blobal 2000. Carter Center, Atlanta, GA.
- 6 THE GLOBAL HEALTH SITUATION. A.O. Lucas, International Health Expert, representing the World Health Organization, Carnegie Corporation, New York, NY.
- 7 MEDICAL CAPABILITIES OF THE U.S. MILITARY. P.K. Russell, representing the U.S. Military, U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.
- 8 THE U.S. CAPACITY TO ADDRESS TROPICAL DISEASE PROBLEMS. K.M. Johnson, Physician-Scientist, representing the U.S. Civilian Sector. National Biological Systems, Rockville, MD.
- 9 THE CONGRESSIONAL RESPONSE. The Honorable Daniel K. Inouye, U.S. Senator from Hawaii (Invited).

SEMINAR ON THE LEGISLATIVE PROCESS: ADVOCACY WORKSHOP
(No abstracts available)

SUMMARY: This seminar will provide a course of instruction on the legislative process, with handouts and reference materials. It will be given by professional lobbyists and Congressional staff.

PRESENTERS: Pat DeLeon Office of Senator Inouye
Margaret Donahue Capitol Associates, Inc.
Congressional Staff Office of Representative Waxman
(To be announced)

SYMPOSIUM: NUTRITION AND INFECTION
(No abstracts available)

- 10 MECHANISMS AND MEDIATORS OF MALNUTRITION AND TROPICAL INFECTIOUS DISEASES. A. Cerami. Rockefeller University, New York, NY.
- 11 VISCERAL LEISHMANIASIS: A MODEL FOR INFECTION-INDUCED CACHEXIA. R.D. Pearson. University of Virginia, Charlottesville, VA.
- 12 DIARRHEA AS A CAUSE OF MALNUTRITION. L. Mata. Institute for Health Research, University of Costa Rica, COSTA RICA.
- 13 MALNUTRITION AS A CAUSE OF DIARRHEA. R.L. Guerrant. University of Virginia School of Medicine, Charlottesville, VA.
- 14 A PRACTICAL STRATEGY FOR THE DEVELOPING WORLD. A.O. Lucas. Carnegie Corporation, New York, NY.

A: MALARIA - BLOOD STAGES

- 15 TROPICAL MEDICINE COMMEMORATIVE FUND LECTURE. K.N. Mendis. University of Colombo, Colombo, SRI LANKA. No abstract available.

- 16 SPECIFIC SUPPRESSION OF PLASMODIUM FALCIPARUM GAMETOCYTEA IN IMMUNE RESIDENTS OF ARSO PIR, IRIAN JAYA

*J.K. Baird, T.R. Jones, B. Leksana, Purnomo, S. Masbar. U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, APO San Francisco 96356-5000

Suppression of gametocytemia in people having naturally acquired immunity to malaria is generally thought to be a product of specific suppression of asexual blood stage parasites. In an epidemiologic study of hyperendemic malaria in Arso PIR, Irian Jaya, we detected evidence which suggests suppression of gametocytemia of P. falciparum is independent of asexual parasitemia. Between November 1987 and March 1988, 240 volunteers submitted to biweekly blood film examination from which the frequency and content of parasitemia was characterized. The study population consisted of volunteers greater than 2 years old and was equally divided between immune natives of Irian Jaya and nonimmune transmigrants from Java. Immune natives positive for P. falciparum proved far less likely to carry gametocytes than nonimmune transmigrants (44% vs. 20%, $p < 0.0001$). Suppression of asexual parasites could not explain this discrepancy because the mean high ring counts of immunes was indistinguishable from nonimmunes ($p > 0.10$). Regression analysis of an IgG gametocyte immunofluorescent antibody test (IFAT) vs. frequency of gametocytemia showed a significant negative regression for the immune natives ($p < 0.01$), but not for the nonimmune transmigrants ($p > 0.25$). In contrast, there was no significant regression of gametocyte frequency vs. an IgG IFAT for asexual parasites in either subpopulation ($p > 0.75$, transmigrants; $p > 0.10$, natives). Antibody mediated suppression of gametocytes independent of immune suppression of asexual blood stages would explain these epidemiologic and serologic observations.

- 17 INFLUENCE OF MAJOR HISTOCOMPATIBILITY COMPLEX GENES ON THE SPECIFICITY OF THE ANTIBODY RESPONSE TO THE P. FALCIPARUM MAJOR MEROZOITE SURFACE PROTEIN. S.P. Chang,* G.S.N. Hui, A. Kato, and W. A. Siddiqui. Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu, HI.

The subunit vaccine strategy for producing a malaria vaccine is based on the ability of single parasite antigens to confer protective immunity in animal models or to induce antibodies which inhibit parasite growth in vitro. Since an effective subunit vaccine must be capable of eliciting immunity in a genetically heterogeneous population, interest has developed in studying the immunogenicity of malaria vaccine peptides and polypeptides in individuals of different genetic backgrounds. While major histocompatibility complex (MHC) control of immune responsiveness to malaria antigens of the sexual stages of the parasite life cycle has been demonstrated, the effect of MHC genes on responsiveness to asexual blood stage antigens had not been examined. A prime candidate antigen for the development of immunity to the asexual blood stages of P. falciparum is the major merozoite surface protein (gp195). The antibody responses to gp195 of congenic mouse strains differing in MHC haplotype are reported in this study. All seven strains of mice were capable of producing gp195-specific antibodies. Generalized immune recognition of gp195 by mice of diverse MHC haplotypes distinguishes gp195 from the P. falciparum circumsporozoite protein and 230,000 and 48,000/45,000 Da zygote surface antigens which are recognized by mice of only a limited number of MHC haplotypes. However, the MHC locus appeared to influence the specificity of gp195-specific antibodies. Immunoblot patterns of mouse sera with parasite antigens were similar for all of the mouse strains, however there were several strains which additionally recognized a few unique fragments or displayed more intense reactivities with specific processing fragments. Congenic mouse strains also differed in their degree of reactivity with two gp195 synthetic repeat peptides. These results suggest that individuals of diverse MHC makeup are capable of recognizing the gp195 antigen, however the recognition of specific gp195 B cell and T cell epitopes may be under MHC gene control. (Supported by USAID)

A: MALARIA - BLOOD STAGES

- 18 THE ROLE OF CONSERVED REGIONS ON THE *P. FALCIPARUM* MAJOR MEROZOITE SURFACE PRECURSOR PROTEIN (GP195) IN PROTECTIVE IMMUNITY. G.S.N. Hui*, L.Q. Tam, S.P. Chang, and W.A. Siddiqui. Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu, Hawaii.

The *P. falciparum* major merozoite surface precursor protein (gp195) is encoded by two (dimorphic) alleles, and the antigenic polymorphism of this protein has been described. *Aotus* monkeys hyperimmunized with purified gp195 are protected from a homologous parasite challenge. In addition, prechallenge sera from these protected monkeys as well as rabbits immunized with the same protein strongly (>90%) inhibit parasites *in vitro*. To determine the relative importance of conserved, allele-specific and variable repeat epitopes in protection, rabbits were immunized with purified gp195/FCA from the FUP (Uganda Palo Alto) or FVO (Vietnam Oaknoll) isolates. Southern blot analysis using allele-specific oligonucleotide probes and restriction enzyme sites showed that FUP and FVC gp195 are members of different allelic groups. In addition, a synthetic oligonucleotide specific for the FUP-gp195 tripeptide repeats did not hybridize to FVO indicating that the variable repeat regions are unrelated. Monoclonal antibody (Mab 5.2) reacted with gp195 from both isolates and was used to affinity purify gp195 from parasite extracts. Mab 5.2 isolated the 195 kDa precursor molecule and processing fragments of 125 kDa, 53 kDa and 43 kDa from the FUP lysates, while the same antibody isolated the 195 kDa molecule and fragments of 93 kDa, 83 kDa, 54 kDa and 41 kDa from the FVO isolate. Antibodies from rabbits immunized with gp195 of one allele showed strong crossreactivity with purified gp195 from the other allele by an ELISA assay, indicating that a large proportion of antibodies are directed against conserved epitopes on both molecules. When sera from these rabbits were used in *in vitro* inhibition assays against parasite of the opposite allele (i.e. FUP vs FVO and vice versa), >70% inhibition was obtained. These results showed that antibodies to conserved epitopes of gp195 are major contributors to parasite inhibition *in vitro*. Conserved regions may therefore play a significant role in gp195-specific protective immunity *in vivo*. (Supported by USAID)

- 19 IDENTIFICATION OF REGIONS OF BLOOD-STAGE MALARIAL PROTEINS THAT MAY COMPRISE VACCINE COMPONENTS.

A.W. Thomas, M. Gross, D.A. Carr, J.D. Chulay and J.A. Lyon. Dept. Immunology, Walter Reed Army Institute of Research, Washington DC 20307-5100 and SmithKline Beckman, King of Prussia, PA 19406-0939.

We have developed a method that allows the identification of regions of blood-stage malarial proteins available to immune attack. A series of overlapping recombinant fragments derived from gp195, the major merozoite surface antigen precursor of *P. falciparum* CAMP strain parasites, were cloned into an expression vector. The expression products were analysed for reactivity with whole immune serum and antibody eluted from immune clusters of merozoites (ICM's), formed when parasites mature in the presence of immune serum (1). In comparison with whole immune serum, a small number of discrete regions of gp195 are recognized by antibodies involved in ICM formation. These regions may represent areas of gp195 available to immune attack on intact parasites. In the light of difficulties encountered in the expression of full length recombinant malarial proteins, and the limitations current technology places on the size of synthetic peptides, recombinant polypeptides comprising these areas of malarial proteins are promising vaccine candidates.

1. Lyon JA., Thomas AW., Hall T. and Chulay JD. Mol. Biochem. Parasitol. (1989) in press.

A: MALARIA - BLOOD STAGES

- 20 IMMUNIZATION TRIALS WITH THE RESA ANTIGEN OF PLASMODIUM FALCIPARUM IN AOTUS MONKEYS. *W.E. Collins, R.F. Anders, T.K. Ruebush, II, D.J. Kemp, G.H. Campbell, and G. Woodrow. Malaria Branch, Centers for Disease Control, Atlanta, GA, USA; Walter and Elisa Hall Institute of Medical Research and Biotechnology Australia, Melbourne, Australia.

The ring-infected erythrocyte surface antigen (RESA) is a candidate for inclusion in a human malaria vaccine. Trials were conducted to test the safety, immunogenicity, and efficacy of synthetic peptides containing repeat sequences of the 3' and 5' regions of the RESA gene product of Plasmodium falciparum, with muramyl dipeptide (MDP) as the adjuvant. Tests also were conducted to compare in Aotus nancymai the immunogenicity and efficacy of recombinant protein Ag632, with Freund's complete and incomplete adjuvant (FCA/FIA) or MDP. Monkeys were immunized with synthetic peptides (EENV)₈, (EDHVEENV)₄ and (DDEHVEETVA)₃ conjugated to diphtheria toxoid and MDP. Maximum serologic responses were obtained with the homologous antigens; no significant differences were observed in maximum parasitemia between experimental and control groups. Monkeys immunized with Ag632 with FCA/FIA or MDP had highest ELISA response to the 11-mer peptide; levels were considerably higher than in the animals immunized with the synthetic peptides. An inverse correlation was found between the ELISA response to the 11-mer peptide and the log of the maximum parasitemias ($r_s = -0.68$, $p = 0.03$). The results of these trials suggest that the immunologically dominant repeat regions (the 4-mer, 8-mer, and 11-mer synthetic peptides and the fused polypeptide Ag632) are by themselves insufficient to induce significant protection against the FUP strain of P. falciparum in A. nancymai. Supported by Vaccine PASA BST-0453-P-HC-2086-06.

- 21 ANTIBODY RESPONSES OF AOTUS MONKEYS IMMUNIZED WITH SYNTHETIC PEPTIDES DERIVED FROM AMINO ACID SEQUENCES OF PLASMODIUM FALCIPARUM BLOOD-STAGE PROTEINS. *G.H. Campbell, T.K. Ruebush II, M.E. Patarroyo, A. Moreno, W.E. Collins, R. Rodriguez, and M. Salcedo. Malaria Branch, Centers for Disease Control, Atlanta, GA, USA, and Instituto de Inmunologia, Hospital San Juan de Dios, Bogota, Colombia.

A combination of 3 synthetic peptides (35.1, 55.1, and 83.1) corresponding to fragments of the 35K, 55K, and 83K bloodstage proteins of P. falciparum has been shown previously to provide protection against challenge in Aotus monkeys. Six monkeys were immunized with 1) the 3 synthetic peptides from source A, independently bound to BSA with glutaraldehyde, and mixed, 2) peptides from source B and treated identically, or 3) a synthetic hybrid polymer composed of the 3 peptides. Fourteen days after the fifth injection, day 84, antibody responses to each peptide were determined in ELISA. Responses to peptides 35.1, 55.1, and 83.1 were found respectively in 6/6, 5/6, and 1/5 monkeys immunized with source A peptides, and in 2/6, 5/6, and 4/6 monkeys immunized with source B peptides. In contrast, 6/6 monkeys immunized with the hybrid polymer produced detectable antibody only to peptide 55.1. Peak responses were obtained in most animals to each peptide after the 4th or 5th immunization. After challenge with P. falciparum FVO parasites no protection was observed in immunized versus control animals. However, in comparative serology of 2 additional trials performed in Colombia in which protection against challenge was observed, 11/11 animals responded to all 3 peptides with the strongest response to the 83.1 peptide. The lack of protective effect in this trial may be due to the absence of strong antibody responses to each of the three peptides. Supported in part by USAID PASA BST-0453-P-HC-2086-06 and WHO Special Programme in Tropical Diseases Research.

A: MALARIA - BLOOD STAGES

22 MALARIAL PARASITES MAY USE DIFFERENT LIPID MODIFICATIONS TO TARGET PROTEINS TO SPECIFIC MEMBRANES.

*R.T. Reese, and H. A. Stanley. The Agouron Institute, La Jolla, CA.

McAb's have been used in either immunofluorescence or immunoelectron microscopic experiments to localize gp185, gp56 and gp34 to the plasma membrane of *Plasmodium falciparum* merozoites. All of these molecules appear to contain the same myristylated glycolipid anchor attached to their carboxyl terminus. On the basis of this type of labeling it appears that gp56 is present in equal or greater concentration than gp185. Gp34 may be less prevalent. A 45 kDa parasite protein has been isolated using McAb's which is associated with a membrane system located between the parasitophorous vacuole membrane and the plasma membrane of the erythrocyte. This molecule, which may be involved in transport, contains palmitic acid, probably attached to the amino terminal portion of the molecule, but is not radiolabelled with myristic acid or certain sugars. A third group of parasite proteins contain myristic acid but are not labeled with either glucosamine or palmitic acid. Preliminary data suggest that this third group of proteins may be exported to the erythrocyte plasma membrane. Thus, three different lipid modifications may be used by *P. falciparum* to direct parasite proteins to different membranes within the host erythrocyte.

23 USE OF A FUSION PEPTIDE AND ANTI-ID ANTIBODIES TO ASSESS THE IMMUNOGENICITY OF A *PLASMODIUM YOELII* SOLUBLE ANTIGEN.

*Sansanee Changkasiri and Diane Taylor, Department of Biology, Georgetown University, Washington, D.C. 20057.

It is well known that parasite antigens circulate in the sera of individuals acutely infected with malaria. The role of these antigens remains unclear. We have identified a heat-stable malarial antigen of M 117,000-130,000 (Py117) that circulates in sera of mice acutely infected with *Plasmodium yoelii*. An analogous antigen circulates in the sera of humans with acute *P. falciparum*. In order to determine if Py117 is involved in immune protection, BALB/c, C3H and C57BL/10 mice were immunized with a 162kd fusion peptide obtained from a *P. yoelii* genomic lambda gt11 library. BALB/c mice were also immunized with monoclonal anti-idiotypic (anti-Id) antibodies (coupled to KLH) that mimic an epitope on Py117. All BALB/c mice immunized with the fusion peptide and anti-Id antibodies developed considerably lower peak parasitemias and a shorter course of infection compared to the adjuvant and KLH controls. All immunized BALB/c mice survived infection. Interestingly, however, B10 and C3H mice immunized with the fusion peptide developed severe courses of infection compared to the controls and a significant number of mice died. These results suggest that a malarial peptide linked to B-galactosidase may cause positive immunoregulation in some strains of mice but suppression in others.

A: MALARIA - BLOOD STAGES

- 24 A NEWLY IDENTIFIED 260 kD PROTEIN OF INTRAERYTHROCYTIC
 PLASMODIUM FALCIPARUM PARASITES CROSS-REACTS WITH THE 11.1
 PROTEIN AND PF155-RESA.

*C. Petersen, R. Nelson, W. Wollish, and J. Leech. San Francisco General Hospital and University of California, San Francisco, CA.

Members of a cross-reacting family of Plasmodium falciparum proteins including 11.1, a megadalton protein of schizont-infected red blood cells (J Cell Biochem S13E:115, 1989); Pf155-RESA, a 155 kD protein of ring-infected red blood cells; and Ag 332, tentatively identified as a 92 kD protein of late stages of asexual parasites (Parasite Immunol 11:15, 1989), have been reported to share amino acid repeat sequences. These repeats are rich in glu-glu dipeptides postulated to be involved in generating serologic cross-reactivity. We report here the identification and characterization of another member of this cross-reacting family, a 260 kD protein of intraerythrocytic parasites.

Human antibodies affinity purified on the 260 kD region of Western blots of trophozoites of Plasmodium falciparum were used to screen a trophozoite-stage lambda gt11 cDNA library. A 1.8 kb clone was identified and human antibodies were affinity purified on the proteins of expressing confluent plaque lifts of the clone. Using this affinity purified antibody and the 1.8 kb clone, the corresponding protein and its gene were investigated. The corresponding protein of MW 260 kD cross-reacts with 11.1 and Pf155-RESA, but is the product of a different gene. The 260 kD protein is Triton X-100 soluble and is variable in molecular weight in different isolates. Immunoprecipitation of ³⁵S-methionine labelled infected red blood cells at ring, trophozoite and schizont stages indicates that the protein is present throughout the intraerythrocytic cycle. The protein is not immunoprecipitated from ¹²⁵I surface labelled infected red blood cells and is thus not the > 250 kD surface labelled antigen associated with cytoadherence. Indirect fluorescent antibody studies using fixed infected red blood cells suggest that the protein is localized to the periphery of the intraerythrocytic parasite.

- 25 SERUM MARKERS OF CELL MEDIATED IMMUNITY IN MALARIA

H. K. Webster*, A. E. Brown, C. Wongsrichanalai, S. Kongchareon.
U.S. Army Medical Component, AFRIMS, Bangkok 10400, Thailand.

Cell mediated immunity (CMI) has an important though poorly understood role in the host response to malaria infection. Direct in vivo measures of CMI are limited. We have used prospective and cross-sectional serum sampling of naturally infected individuals to examine three indirect measures of T-cell activity: TAC antigen (TAC), CD8 protein (CD8) and interferon-gamma (IFN). Each T-cell marker was determined by immunoassay using commercial kits and values expressed as activity units per ml. Serum marker positivity (mean + 2SD) was based on values for Bangkok controls. Symptomatic Thai soldiers with acute falciparum malaria (n=75) were studied at the time of diagnosis and through treatment and convalescence. On admission, serum elevations were found to TAC (71/75), CD8 (47/75) and IFN (52/75). Thirty-four patients were positive and 1 negative for all three assays. TAC and CD8 remained high for about 7 days and then decreased to basal levels. IFN elevations were mostly observed on admission day. TAC levels tended to peak prior to those of CD8. In an endemic group of aparasitemic Thai villagers (n=33) 11 of 33 had elevated levels of TAC or IFN. However, 29 of 33 were positive for CD8 with 8 of 29 positive for both CD8 and IFN. These observations suggest massive T-cell activation kinetically coincident with large-scale suppressor cell activity during acute infection and, to a lesser degree, in aparasitemic endemic villagers. These events may underly the immunosuppression of falciparum malaria and contribute to retardation in the development of both humoral and cellular protective responses in malaria.

B: VIROLOGY - DIAGNOSIS

- 26 A GENERAL PROCEDURE FOR PRODUCTION OF VIRAL ANTIGENS FOR ENZYME IMMUNOSORBENT ASSAY FROM INFECTED TISSUE CULTURE CELLS. T.G. Ksiazek, F.R. Bethke, and J. Smith. USAMRIID, Ft. Detrick, Frederick, MD.

A simple, practical, and inexpensive means of producing viral antigens for use in enzyme immunosorbent assays is described. The method, which utilizes non-denaturing, non-ionic detergent, produces viral antigens which directly coat microtiter plates at dilutions greater than 1:1000. Homologous hyperimmune mouse ascitic fluids, when reacted with antigen-coated plates, result in high titers ($>10,000$) and high optical density values (>1.5) at the lower range of dilutions. Human convalescent sera from persons infected or vaccinated with one of several viruses, when reacted with plates coated with antigens produced by this method, also yield substantial titers and optical density values. The same ascitic fluids or sera, when reacted with control antigens consisting of similar extracts of normal cells, give negligible OD values. The antigen extraction method has been applied successfully to viruses of the families Togaviridae, Flaviviridae, Arenaviridae, Rhabdoviridae, Bunyaviridae and Reoviridae.

- 27 APPLICATION OF ANTIGEN CAPTURE ENZYME IMMUNOASSAY FOR THE DETECTION OF WESTERN EQUINE ENCEPHALITIS VIRUS (WEE) IN MOSQUITO POOLS.

P.A. Weber* and J.L. Hardy. School of Public Health, University of California at Berkeley, Berkeley, CA.

WEE continues to be a veterinary and public health problem in California because the virus is maintained in nature by a transmission cycle involving mosquitoes and wild vertebrate hosts, and potentially can be introduced into equine and human populations each summer. Thus, a rapid and sensitive surveillance system is required to detect viral activity in mosquito populations before equines and humans become involved in the transmission cycle. Therefore, an antigen capture enzyme immunoassay (EIA) was developed to detect WEE virus in mosquito pools. This assay employs two anti-WEE monoclonal antibodies to capture virus from the sample and one biotinylated anti-WEE monoclonal antibody to detect captured virus. After reacting with streptavidin/alkaline phosphatase an amplified substrate system (ELISA Amplification System, BRL, Inc., Gaithersburg, MD) is used to detect bound enzyme. The level of sensitivity is 100-200 pg of viral protein using purified virus, and $10^{4.2}$ PFU per ml of virus in individual *Culex tarsalis* females infected by intrathoracic inoculation. This can readily detect WEE virus in mosquito pools containing one infected and 49 normal females, provided the virus titer was $10^{3.8}$ PFU/ml. It is concluded that this test may be less sensitive, but more rapid than mouse inoculation and the *in situ* EIA for detection of virus in naturally infected mosquito pools. This conclusion is being evaluated currently by retrospective and prospective studies on field collected and coded mosquito pools. A similar antigen capture EIA, developed for SLE virus by Dr. T. Tsai (Centers for Disease Control, Ft. Collins, CO) is being evaluated concurrently.

B: VIROLOGY - DIAGNOSIS

28 EVALUATION OF ENZYME IMMUNOASSAY FOR DIAGNOSIS OF EASTERN EQUINE ENCEPHALITIS IN EQUINES IN FLORIDA.

*J.G. Olson, E.P.J. Gibbs, B. All, H.L. Rubin and J.H. Wilson. U.S. Naval Medical Research Unit No.3, Cairo, Egypt, University of Florida, Gainesville, FL and Animal Disease Diagnostic Laboratory, Kissimmee, FL.

Enzyme immunoassays (EIA) were developed to measure IgM and IgG classes of antibody to eastern equine encephalomyelitis (EEE) virus in equines. Assays were evaluated for correlation with standard serologic tests for laboratory confirmation of EEE viral infections and for measurement of immune status in equines. Material tested included 305 sera from equines submitted to the Florida State Veterinary Diagnostic Laboratory with clinical diagnoses of encephalopathy, and 495 sera collected from 67 foals that were immunized with EEE vaccine and tested repeatedly to measure the immune response. The ability of the IgG EIA to confirm clinically diagnosed EEE infections was 88% sensitive and 85% specific when compared to the hemagglutination inhibition (HI) test for demonstrating 4-fold rises in antibody titers from the acute to the convalescent phase of illness. The IgM EIA was 94% sensitive and 86% specific when acute and convalescent phase sera were tested. When only the acute phase serum was tested the IgM EIA was 71% sensitive and 77% specific. Among the 305 sera submitted to the laboratory for testing, the correlation between IgG EIA and HI antibody titers was highly significant ($r^2=0.78$, $p<0.001$). Among the 495 sera tested following the administration of EEE vaccine, correlations between IgG EIA titers and HI and neutralizing antibody titers were highly significant ($r^2=0.71$, $p<0.001$) and ($r^2=0.68$, $p<0.001$), respectively. Only 2 of the 495 sera tested by IgM EIA showed evidence of IgM class antibody to EEE in response to immunization. (Supported by NAVMEDRSCHDEVCOM, Bethesda, MD, Work Unit No. 3M162770A879.AA.122)

29 SENSITIVITY OF IGM ELISA AND HI IN PATIENTS WITH DENGUE INFECTION CONFIRMED BY VIRUS ISOLATION.

E.B. Hayes, D.G. Gubler, I.G. Gomez, G.S. Sather. San Juan Laboratories, Centers for Disease Control, GPO Box 4532, San Juan, Puerto Rico.

IgM ELISA is increasingly used as a diagnostic test for dengue despite limited published data on sensitivity and specificity of this test. We retrospectively compared the sensitivity of an IgM capture ELISA with the hemagglutination inhibition (HI) test in detecting dengue infection in 131 patients with dengue confirmed by virus isolation. Sensitivity of IgM ELISA of sera from 68 patients using a mixture of all four dengue serotype antigens, and 63 patients using the four antigens separately was similar. The infecting serotype was indicated correctly by separate antigen testing in only 30 (59%) of 51 samples positive by IgM ELISA to at least one antigen. The HI test showed a 4-fold or greater titer rise to at least one dengue antigen in 130 (99%) of 131 serum pairs. When convalescent samples were obtained 7-20 days from onset ($n=76$), IgM ELISA sensitivity was 96% (95% C.I. = 89-100%), not statistically different from that of HI (100%, $p=0.25$). By 60 days IgM ELISA sensitivity had decreased to 30% (95% C.I. 7-65%). Sensitivity of IgM ELISA was similar in primary and secondary infections as determined by WHO criteria. IgM ELISA using mixed dengue antigens is efficient and highly sensitive in detecting dengue infection if samples are obtained 7-20 days after onset of illness.

B: VIROLOGY - DIAGNOSIS

30 DEVELOPMENT OF AN IMMUNOSORBENT-BASED FILTER HYBRIDIZATION ASSAY FOR THE DETECTION OF RIFT VALLEY FEVER VIRUS RNA.

F.K. Knauert* and B.A. Farrish. Disease Assessment Division, USAMRIID, Fort Detrick, Frederick, MD 21701-5011.

The presence of excessive amounts of nonspecific, contaminating material in clinical samples, such as serum, decreases the sensitivity and precision of filter hybridization assays by reducing the efficiency of filtration, and the availability of binding sites for immobilization of specific target molecules. We have tried a number of modifications to a standard sample preparation procedure and have found the most effective method to be a adaptation of the procedure of Jansen, et al. (J. Clin. Microbiol. 22:984, 1985). Sample is introduced into microtiter wells coated with a mixture of Rift Valley fever virus (RVFV)-specific monoclonal antibodies. The sample is removed, the wells are extensively washed, and the "captured" virus-specific particles are subsequently eluted and prepared for filter hybridization by standard methods. We have tried a number of different elution buffers, and have found that a solution of proteinase K is the most specific and efficient. The modified procedure is at least as sensitive as our standard procedure used to prepare "simple" samples such as infected cells or inactivated RVFV vaccine, which we use as a basis of comparison to evaluate the effectiveness of different modifications, enabling us to detect less than the equivalent of 5×10^3 PFU of RVFV. In addition to increasing the sensitivity and precision of our procedure, this method allows us to take advantage of microtiter plate sample handling technology, enabling us to handle a larger number of samples in the same time period. Experiments are underway to apply this modified procedure to other sample types, such as tissue homogenates, which in the past have been difficult to prepare optimally for filter hybridization.

31 DIAGNOSIS OF TICK-BORNE VIRUS INFECTIONS USING THE POLYMERASE CHAIN REACTION.

V.K. Ward, L.D. Jones, M.A. Morse and P.A. Nuttall.* NERC Institute of Virology & Environmental Microbiology, Mansfield Road, Oxford, OX1 3SR, U.K.

The sensitivity and specificity of the polymerase chain reaction (PCR) was investigated for the diagnosis of Dugbe (Nairovirus, Bunyaviridae) or Thogoto (unclassified) virus infections in ticks and laboratory animals. The first step involved synthesis of cDNA from purified viral RNA using reverse transcriptase. The product (first strand cDNA) was used in a PCR containing two DUG or THO specific primers, nucleotides, and Tag polymerase. Multiple cycles of heat denaturation, reannealing and extension resulted in amplification of the cDNA. The amplification product was detected by dot blot or agarose gel electrophoresis, and the specificity confirmed by hybridization with DUG or THO viral cDNA. Using this method, DUG viral RNA was detected in the equivalent of 1/200 of an infected tick. Application of this technique to the diagnosis of infections in laboratory animals is now being investigated, and the results compared with biological (plaque assay, mouse inoculation) methods of detecting virus infections.

3: VIROLOGY - EPIDEMIOLOGY

- 32 INAPPARENT JUNIN VIRUS (JV) INFECTION AMONG RURAL MALES IN AREAS ENDEMIC FOR ARGENTINE HEMORRHAGIC FEVER (AHF). J.G. Barrera Oro, J.I. Maiztegui, C. Saavedra, B.G. Mahlandt, S. Levis, J. Spisso, E. Tiano, M.R. Feuillade, and K.T. McKee, Jr. The Salk Institute (CSD), Frederick, MD; INEVH, Pergamino, Argentina; and USAMRIID, Ft. Detrick, Frederick, MD.

Published estimates of the prevalence of inapparent JV infection in Argentina are based upon limited geographic and numeric samples. The conduct of a placebo-controlled vaccine study in southern Santa Fe Province (1 of 2 provinces with highest AHF incidence at present) afforded an opportunity to assess the prevalence (and, ultimately, incidence) of inapparent JV infections in a large geographic area among individuals most at-risk for disease: namely, adult males living or working in rural areas. Approximately one third (6494) of the population of at-risk adult males from a 41-county region of Santa Fe Province were recruited to participate in the vaccine study. Screening sera were obtained from each volunteer were tested for JV antibodies (Ab) using a complement-enhanced plaque-reduction neutralization (PRN) test. JV PRN Ab was found in 201/6494 (3.1%) volunteers from the 41-county area; prevalence by county varied from 0% to 8.5%. However, 38/2102 individuals (1.8%) from 12 counties along the northern border of the study region had demonstrable JV Ab, while 64/2023 (3.2%) individuals from 14 counties in the middle of the study area and 99/2369 (4.2%) from those along the southern border were positive ($p < .001$). Relationships among these findings, demographic factors, and progressive extension of the AHF-endemic zone, will be discussed.

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RISK FACTORS FOR CRIMEAN-CONGO HEMORRHAGIC FEVER IN RURAL NORTHERN SENEGAL. Louisa F. Chapman*, Mark L. Wilson, Bernard LeGuennou, and Susan P. Fisher-Hoch. Special Pathogens Branch, DVRD, CID, Centers for Disease Control, Atlanta, Ga. 30333; Institut Pasteur, B.P. 220, Dakar, Senegal; and USAMRIID, Fort Detrick, Frederick, MD. 21701.

Crimean-Congo Hemorrhagic Fever (CCHF) is a tick-borne viral zoonosis widespread throughout Eurasia and Africa. Little is known of interepidemic transmission to humans, although vector bites and contact with sick vertebrates have been suspected. Isolation of CCHF virus from ticks and evidence of antibodies in humans and animals from the Sahel region of north-central Senegal suggested recent transmission. We undertook a retrospective cohort study there to determine risk factors for CCHF infection among the Fulaar, semi-nomadic sheep and cattle herders. From about 1,000 persons living within a 10 km. circumference of the village of Yonofere, more than 300 residents of 37 camps, age 5 or older, were bled and questioned. Putative exposure risks such as animal and animal waste contact, tick exposure, human and animal health care, meat handling, scarification and funeral activities were sought through questionnaire. Personal health histories of symptoms suggestive of CCHF during the past two years were obtained. Anti-CCHF IgG antibodies were found in more than 8% of persons studied, and were similar for males (7.5%) and females (8.7%), and among age groups. Two IgM positives were found. Infections appeared to be clustered, as most of the 37 camps were negative while 8 with a sero prevalence of at least 20% accounted for 2/3 of all positives. Analysis of exposure variables suggests possible mechanisms of infection.

B: VIROLOGY - EPIDEMIOLOGY

- 34 EXAMINATION OF SERA COLLECTED BY THE HEMORRHAGIC FEVER COMMISSION DURING THE KOREAN CONFLICT FOR ANTIBODIES TO HANTAVIRUSES. James W. LeDuc, Thomas G. Ksiazek, and C. A. Rossi. USAMRIID, Fort Detrick, Frederick, MD.

Hantaviral infections were first encountered by American clinicians during the Korean Conflict, when U.S. forces suffered from a mysterious "new" hemorrhagic fever. Although all attempts to isolate the causative agent failed, the team of scientists investigating this disease systematically preserved serum samples from about 10% of those infected. This collection of sera still exists, and we recently examined these sera for antibodies to hantaviruses. Sera were obtained from 245 patients, and an average of three sera were drawn from each patient, usually on admission to hospital, at about 1 week post admission, and sometime later. We tested all sera drawn from each patient for IgM- and IgG-specific antibodies to Hantaan virus by an enzyme immunoassay, and at least one sample from each patient by plaque-reduction neutralization test (PRNT) to both Hantaan and Seoul virus to identify the infecting virus. We found that only 15 patients admitted to the study failed to develop specific anti-hantaviral antibodies. Most sera contained high-titered, IgM-specific antibody on admission, and by day 7 post onset, all patients were IgM seropositive. IgG antibody appeared somewhat later in disease, and titers increased less rapidly than IgM. All attempts to detect hantaviral antigen were unsuccessful. All seropositive patients had highest PRNT titers to Hantaan virus, suggesting that this virus was responsible for the disease seen. These results confirm that the hemorrhagic fever described during the Korean Conflict was in fact due to Hantaan viral infection, and further demonstrate that measurement of specific IgM antibody is the method of choice for diagnosis of acute disease.

- 35 RAT AND MOUSE CONTACT AND INFECTION WITH TWO RODENT-BORNE VIRUSES IN AN URBAN POPULATION FROM BALTIMORE, MD. J.E. Childs, G.E. Glass, T.G. Ksiazek, C.A. Rossi, and J.W. LeDuc. Johns Hopkins University, Baltimore, MD and USAMRIID, Frederick, MD.

We obtained questionnaire data and serum samples from 1180 persons visiting a sexually transmitted disease clinic in Baltimore from 1986-1988. Information on rat and mouse sightings and contact was obtained, and sera were tested by ELISA and plaque reduction neutralization assay for antibodies to Seoul (SEO) virus, a rat-borne Bunyavirus (genus: Hantavirus), and LCMV, a mouse-borne Arenavirus. The study population was primarily African-American (94%) and male (70%), with a median age of 23.5 years. House mice (Mus musculus) were reported within residences far more frequently than rats (Rattus norvegicus; 48.8% and 5.9% of respondents, respectively), although rats were more commonly sighted on the streets or in alleys (63.8% vs. 32.1% for mice). Mice were also more commonly encountered in the work place (22.8% vs. 13.7% for rats). Reported contact with rodents was higher for mice than rats with regard to trapping (49.1% and 19.8%, respectively), contact with feces (30.8% and 13.0%) and picking up an animal (10.4% and 4.8%), although bites were similar (0.9% and 1.0%). Antibody to a Baltimore isolate of SEO virus was found in 3 individuals (0.3% of those tested), while 54 (4.7%) had antibodies to LCMV. All of the SEO positives were African-American males, while all races and both sexes were infected at similar rates with LCMV. There were no obvious associations between seropositivity and reports of rodent sightings or contact. The data indicate higher levels of exposure to house mice and LCMV than to rats and SEO virus in Baltimore.

B: VIROLOGY - EPIDEMIOLOGY

- 36 PROSPECTIVE EPIDEMIOLOGICAL STUDY ON DENGUE INFECTION OF U.S.
PEACE CORPS VOLUNTEERS STATIONED IN THE PHILIPPINES
*C.G. Hayes, T.F. O'Rourke and A. R. Sarr. US. Naval Medical
Research Unit and U.S. Peace Corps, Manila., Republic of the Philippines

Over 250 U.S. Peace Corps volunteers are stationed in the Republic of the Philippines. These volunteers enter the country in small groups that stay together for several weeks of initial training. Afterwards, they disperse to their individual work sites which are scattered throughout the country. The volunteers usually remain in the country for 2 years. Since 1983 we have been serologically monitoring the volunteers to detect infection with dengue viruses. A baseline serum is obtained shortly after arrival, a mid-tour sample is taken after 1 year and a final sample shortly before departure. In addition, if any of the volunteers developed an acute febrile illness and returned to the Peace Corps Medical Unit in Manila for consultation, acute and convalescent samples were obtained. Interview forms were sent to all individuals diagnosed positive for dengue to obtain epidemiological and clinical data. To date over 800 volunteers have been enrolled in this study. Among those individuals that have been in country for >1 year, about 21% have had a dengue infection documented serologically (HI test or IgM antibody capture ELISA). This study has shown that dengue is a common infection among the volunteers, and is widespread geographically in the country. Most of the infections have resulted in an illness typical of classical dengue fever even among those volunteers who have experienced more than one dengue infection during their two year tour. In addition to the dengue infections, chikungunya fever also was diagnosed in some volunteers during an epidemic that swept through the Philippines during 1985-86, and this data also will be presented.

- 37 SENSITIVITY AND SPECIFICITY OF CLINICAL CASE DEFINITIONS FOR DENGUE
FEVER
*S.H. Waterman, D.J. Gubler, G.E. Sather, and R. Bailey. Los Angeles
County Department of Health Services, Los Angeles, CA and Centers
for Disease Control, San Juan, PR.

We evaluated the usefulness of 6 clinical case definitions for dengue fever in 137 persons who were ill or had confirmed dengue infection from a population-based epidemiologic investigation during a classic dengue 4 outbreak in two Puerto Rican communities in 1982, and in 38 persons with acute febrile illness clinically studied during a classic dengue 1 outbreak in Maceio, Brazil in 1986. Confirmed cases in Puerto Rico (n=59) were defined as persons with a 4-fold rise in dengue HI antibody titer on paired serum specimens. Dengue diagnosis was confirmed in Brazil by virus isolation and/or positive IgM ELISA on a single acute serum specimen (n=25). Using a common case definition of fever and headache plus at least one other symptom including eye pain, myalgia, arthralgia and rash, the case definition sensitivities in Puerto Rico and Maceio, respectively, were 39% (95% CI=33, 45) and 92% (87, 97); specificities were 47% (41, 53) and 17% (6, 28). Frequent asymptomatic infections in the Puerto Rican survey lowered considerably sensitivities of case definitions in Puerto Rico. The highest specificities (and lowest false positive rates) in both populations were obtained with a case definition requiring fever, headache, and three of the above symptoms, but corresponding sensitivities declined sharply. These data suggest, as does a study by Dietz, et al., from Rio de Janeiro in 1986, that surveillance and epidemiologic investigations of classic dengue outbreaks will suffer from significant bias unless they are laboratory based.

38 **MICE HOMOZYGOUS FOR THE MUTATION 'SEVERE COMBINED IMMUNODEFICIENCY' (*SCID*) SUPPORT THE GROWTH, MATURATION AND DEVELOPMENT OF INFECTIVE LARVAE OF THE HUMAN FILARIAL PARASITE, *BRUGIA***

MALAYI. Rajan, T.V.*~, Greiner, D.L.~, and Shultz, L.D.# (~Department of Pathology, University of Connecticut Health Center, Farmington, CT 06032, and #Jackson Laboratory, Bar Harbor, ME 04609).

The ability of athymic *nu/nu* mouse to support the growth of the L3 larvae of *Brugia malayi* prompted us to investigate whether *scid/scid* mice would similarly be able to support L3 growth. C.B. 17-*scid/scid* mice lack the recombinase responsible for the assembly of immunoglobulin and T cell receptor genes and as a consequence contain no functional T or B lymphocytes. C.B. 17-*scid/scid* mice were raised in the laboratory of LS and young adults were injected i.p. with 100 *B. malayi* L3 larvae each. Three mice were sacrificed two months following the injection and examined grossly and by peritoneal lavage for the presence of worms. We observed a large number of adult stages of the worm, ranging from young adults with no obvious reproductive cells to mature adults. In addition, lavage fluid contained motile microfilariae (mf). Four other mice were carried for another two weeks and examined by lavage alone. The mice survived the lavage procedure and all lavage fluids contained numerous motile mf. These results indicate that *scid/scid* mice may be a valuable small rodent model for the maintenance of *B. malayi* stocks. An additional attractive feature of this mutant mouse is that it supports the growth and maturation of human T lymphocytes when reconstituted with human thymus tissue or fetal liver cells, which might permit one to study the development of human lymphocytes specifically immune to filarial parasites. We are now investigating whether *Onchocerca lienalis* L3 will grow in these mice and whether mice doubly mutant at the *scid* and *beige* (*be*) loci offer any advantages over the *scid/scid* mice.

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CYTOKINE REGULATION OF IgE PRODUCTION IN HUMAN FILARIASIS.

*C.L. King, E.A. Ottesen, and T.B. Nutman. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

To define the immunoregulatory mechanisms underlying the elevated IgE serum antibody found in patients with filariasis we studied polyclonal IgE production by peripheral blood mononuclear cells (PBMC) from 5 amicrofilaremic patients with *Loa loa* infection, with special attention to the role of interleukin-4 (IL-4) and interferon-gamma (IFN- γ) in the generation and regulation of the response.

Spontaneous in vitro IgE production was elevated in all patients (561-5,845 μ g/ml; normals, undetectable) and was correlated with individual serum IgE levels (500-9,270 ng/ml; $p < 0.01$). In the three patients so studied addition of filarial parasite antigen (0.01 to 0.001 μ g/ml) to PBMC cultures significantly stimulated polyclonal IgE production (145% to 400% of unstimulated levels). The essential role of IL-4 in the generation of this response was demonstrated when simultaneous addition of anti-IL-4 completely inhibited antigen stimulated IgE production. An inhibitory role of endogenously produced IFN- γ was indicated when the addition of anti-IFN- γ to the cultures augmented filarial antigen stimulated IgE production by 33-82%. Furthermore, addition of recombinant human IFN- γ to PBMC from all 5 patients inhibited spontaneous in vitro IgE production in a dose dependent fashion (35 \pm 8% inhibition at 10 U/ml IFN- γ ; 82 \pm 2% inhibition at 10⁵ U/ml IFN- γ).

This study demonstrates that filarial antigen-stimulated IgE production in patients with loiasis is mediated by IL-4 and downregulated by IFN- γ and that the amount of IgE produced depends on the relative quantity of IL-4 and IFN- γ generated by parasite specific T cells. A difference in the balance between IL-4 and IFN- γ production may explain the the variation in serum IgE levels seen in patients with different clinical manifestations of this infection.

- 40 REGULATION OF HELMINTH-INDUCED EOSINOPHILIA: INDUCTION OF INTERLEUKIN-5 mRNA. *A.F. Limaye, E.A. Ottesen, and T.B. Nutman. Laboratory of Parasitic Diseases, NIH, Bethesda, MD.

The eosinophil has been implicated in both resistance and pathogenesis of helminth infections, and in other systems interleukin-5 (IL-5) has been identified as a critical component for the development of eosinophilia. In order to understand the regulation of eosinophilia in human helminth infections, we examined the role of IL-5 in filaria-induced eosinophilia. Using total RNA isolated from peripheral blood mononuclear cells (PBMC) of normal and filaria-infected individuals, we showed by Northern blot analysis with an IL-5 specific probe that IL-5 mRNA was induced after stimulation with phorbol myristate acetate (PMA) and the calcium ionophore A23187 (Iono) at 50ng/ml and 1µg/ml respectively. A 0.91kb transcript representing the IL-5 mRNA was first detected at 4hr, reached a maximum at 6hr, then progressively declined. Densitometry revealed that the relative amount of IL-5 mRNA induced by PMA/Iono stimulation of the PBMC *in vitro* was clearly correlated with the eosinophil number present in each individual's peripheral blood. Most importantly, stimulation of filaria-infected but not normal PBMC with soluble *B. malayi* antigen (10µg/ml) induced IL-5 mRNA, with kinetics different from that of the mitogen-driven cultures. Induction occurred 48hr after stimulation with an antigen response similar to that seen for parasite antigen-specific T-cell proliferation. This study demonstrates that filaria-infected eosinophilic individuals produce IL-5 mRNA in response to parasite antigen and provides a system for examining the molecular events involved in parasite antigen-T cell interactions.

- 41 HUMAN FILARIAL PARASITES SYNTHESIZE PROSTAGLANDINS.
*LX Liu, PF Weller. Beth Israel Hospital, Harvard Medical School, Boston, MA.

Mechanisms of pathogenicity and immune modulation in human filariasis are poorly understood. Microfilariae are circulating multicellular parasites that must interact with host leukocytes, platelets, and endothelium. Bloodborne microfilariae are also exposed to plasma arachidonic acid, a fatty acid substrate for prostaglandin (PG) synthesis. To determine if microfilariae can utilize exogenous arachidonic acid to form prostaglandins, 10⁶ microfilariae of the human filarial parasite *Brugia malayi* were incubated with ³H-arachidonic acid [15nM] for 30 min, followed by microfilarial lipid extraction and resolution by reverse phase HPLC. Two ³H peaks eluted as PGE₂ and 6-keto-PGF_{1α}, the stable breakdown product of prostacyclin. TLC and radioimmunoassay confirmed the formation of PGE₂ and 6-keto-PGF_{1α}. Minimal amounts of these compounds were formed by ultrasonicated or cooled (4°C) microfilariae, and none by boiled microfilariae, indicating that these compounds were enzymatically formed. Prostaglandin production was not significantly inhibited by pre-treating microfilariae with the mammalian cyclooxygenase inhibitors indomethacin [1-10µM] or aspirin [0.1-2mM].

These findings indicate that microfilariae of *Brugia malayi* can synthesize prostaglandins from exogenous arachidonic acid. Parasite production of prostacyclin may allow circulating microfilariae to prevent platelet aggregation onto their surfaces, while PGE₂ may mediate immune defects seen in patients with filariasis. The biosynthesis of prostaglandins by bloodborne nematodes may constitute a newly recognized mechanism of parasite-host interactions and parasite pathogenicity.

C: FILARIASIS - IMMUNOREGULATION

- 42 HOST RESPONSES TO BRUGIA PAHANGI INFECTION IN JIRDS BORN TO BRUGIA INFECTED MOTHERS. S.C. Bosshardt*, C.S. McVay, S.U. Coleman, and T.R. Klei. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

Altered immunity to filarial infections in offspring of Brugia-infected female jirds was studied by inoculating 100 B. pahangi L₃ into age-matched male progeny of infected and uninfected mothers. Infections in 4 week old offspring resulted in mean adult worm recoveries of 14.7 ± 10.6 and 13.4 ± 10.2 in filariae-exposed and unexposed offspring, respectively. The mean number of intralymphatic thrombi per adult worm did not differ in offspring from infected ($\bar{X} = 2.0 \pm 2.8$) or uninfected females ($\bar{X} = 1.5 \pm 2.0$). Infections in 2 week old offspring yielded similar results. Pulmonary inflammatory responses of Brugia-exposed offspring infected at 2 weeks of age were significantly reduced ($p \leq 0.02$) around both Brugia antigen and diethanolamine coated beads compared to infected offspring from normal mothers. Significantly lower IgG antibody titers ($p \leq 0.05$) to adult B. pahangi antigens were measured by ELISA in the sera of infected, filariae-exposed offspring when infected at 4 weeks of age. Serum antibody titers to Brugia measured in offspring infected at 2 weeks of age were similar. Western immunoblot analysis indicated both qualitative and quantitative reductions in serum IgG antibody recognition of adult B. pahangi antigens in infected offspring of both ages. These results contradict earlier reports by indicating no significant alterations of offspring immunity to homologous infection as measured by either adult worm recoveries or lymphatic lesion severity. However, reduced pulmonary granulomatous reactivity and IgG antibody responses in offspring from infected mothers suggests that some maternal immunoregulation of offspring immune responses occurs. Supported by WHO grant #870049.

- 43 IN VITRO B AND T CELL RESPONSIVENESS TO RECOMBINANT FILARIAL ANTIGENS. D.L. Ellenberger*, N.J. Pieniazek, M.L. Eberhard, and P.J. Lammie. LSU Medical Center, New Orleans, LA; Emory University, Atlanta, GA; and Parasitic Diseases Branch, CDC, Atlanta, GA.

Little is known about the recognition of specific filarial antigens or the clinical consequences of immune responsiveness to specific peptide antigens. Sufficient quantities of purified antigens to conduct these studies can not be generated by conventional means. Therefore, a lambda expression library was constructed from adult Brugia pahangi and recombinant clones were isolated by screening the library with sera from patients living in an area endemic for Wuchereria bancrofti. Two recombinant clones, KL and 1112, stimulate peripheral blood lymphocytes from 7/24 and 13/25 Haitian subjects, respectively, with net proliferative reactivity ranging from 3,900-31,700 cpm. Significant in vitro antibody production was detected in KL stimulated cultures from 4/13 subjects and in 5/13 1112 stimulated cultures. Optimal in vitro antibody production was observed at 10^2 - 10^4 fold lower concentration of antigen than were required for optimal proliferative responsiveness. Since these antigens elicit both humoral and cellular responses, additional studies with KL and 1112 derived peptides will permit epitope mapping and may shed light on the heterogeneity of host B and T cell responsiveness to specific filarial antigens. (Supported in part by NIH grant AI-24459).

- 44 MOLECULAR DEFINITION OF A T CELL EPITOPE IN HUMAN LYMPHATIC FILARIASIS. T.B. Nutman, P. Arasu, N. Raghavan, D.O. Freedman, V. Kumaraswami, K. Jayaraman, F.B. Perler. National Institutes of Health, Bethesda, MD, New England Biolabs, Beverly, MA, and Anna University, Madras, India.

The study of cellular immune responses to filarial infections has in part been complicated by a lack of defined parasite antigens. To circumvent this problem and, at the same time, to examine antigens responsible for the induction of T cell responses in this infection, purified recombinant proteins were used to stimulate peripheral blood mononuclear cells (PBMC) from individuals with filarial diseases. One of several cloned *Brugia malayi* (Bm) antigens derived from a λ gt11 genomic Bm expression library, termed λ Bm19, was found to induce proliferation of human T cells in a parasite-specific, antigen dose-dependent manner. The λ Bm19 DNA sequence hybridized to a 3.2 kb transcript on Northern analysis and has been localized to the developing uterine microfilariae by *in situ* hybridization. The sequence of the insert predicts an open reading frame of 154 amino acids (~ 16 kD) without sequence or protein homology to other known genes or gene products. Five non-overlapping peptides were then synthesized from consensus areas defined by 2 different predictive models of T cell epitopes and used to stimulate PBMC from 8 individuals with chronic filarial lymphatic obstruction. 6/8 responded to the entire purified λ Bm19 fusion protein (stimulation indices [SI] from 3-10). Most importantly, each of these 6 only reacted (SI from 2.3 to 8.4) with a peptide corresponding to amino acids 24-37 and did so in a dose-dependent manner. In marked contrast, there was no response to any of the other 4 peptides. Thus, the predominant T cell epitope of a defined Bm molecule has been mapped and should help clarify the role of antigen structure in the induction of the cellular immune responses seen in lymphatic filariasis.

- 45 IgG3 ANTIBODY REACTIVITY TO A CLONED *BRUGIA* ANTIGEN CORRELATES WITH AMICROFILAREMIC DISEASE-FREE STATUS IN HUMANS WITH LYMPHATIC FILARIASIS *J.W. Kazura, P. Maroney, K. Forsyth, M. Alpers, and T. Nilsen. Case Western Reserve Univ., Cleveland, OH and Papua New Guinea Institute of Medical Research, Goroka.

Examination of immune reactivity to putatively protective filarial antigens in humans with naturally occurring acquired resistance is important in defining the potential utility of such molecules for a vaccine. We describe the capacity of a cloned protein corresponding to a 62K *Brugia malayi* antigen (r62) to induce resistance to microfilaremia in mice and IgG subclass antibody (ab) reactivity to r62 in 54 disease-free residents of an endemic area of *Wuchereria bancrofti* infection in Papua New Guinea. Immunization of mice with 2 ug r62 (expressed in pATH 3 vector) resulted in 40-58% reduction in parasitemia compared to control animals given control protein (3 experiments, $p < 0.001$). Human subjects were divided into 4 groups: putatively resistant amicrofilaremic (mf-) adults, microfilaremic (mf+) adults (mean age 25 years); mf+ and mf- children (mean age 4 years). With respect to IgG4 ab, mf+ children had significantly higher mean levels than mf- children, mf+ adults, or mf- adults (1014 vs 324, 223 and 216 ug/ml, respectively, $p < 0.001$). There were no differences ($p > 0.05$) among the groups with respect to IgG1 ab (range of means 34-77 ug/ml) or IgG2 ab (undetectable levels). In contrast, IgG3 ab to r62 was lower ($p < 0.001$) for mf- adults compared to the other groups (4.6 vs 17.7 to 47.5 ug/ml). There were no differences among the groups with respect to IgG subclass abs to whole *B. malayi* extract. These data suggest that IgG3 ab reactivity to r62, a recombinant antigen with demonstrated protective efficacy in mice, may be a marker of acquired resistance to microfilariae in humans.

C: FILARIASIS - IMMUNOREGULATION

- 46 ANALYSIS OF ISOTYPE-SPECIFIC ANTI-FILARIAL ANTIBODIES IN A HAITIAN PEDIATRIC POPULATION. W.L. Hitch*, P.J. Lammie, and M.L. Eberhard. Dept. of Parasitology, LSU Medical Center, New Orleans, LA; Parasitic Diseases Branch, CDC, Atlanta, GA.

Previous studies of anti-filarial IgG levels in a Haitian pediatric population have demonstrated age-related shifts in anti-filarial immunity. This study was undertaken to examine levels of isotype-specific anti-filarial antibodies. Serum samples were collected by finger-prick from more than 100 pediatric subjects (age 1-15 yrs). Isotype-specific anti-filarial antibody levels were determined by ELISA using mouse monoclonal antibodies directed against human IgG subclasses. Preliminary analysis of antibody levels in 51 subjects (10 microfilaremic, 41 amicrofilaremic) reveals differences in the distribution of isotype specific antibody levels as well as age related variations. The anti-filarial antibody response was predominantly of the IgG1 and IgG4 subclasses. When endpoint titers were analyzed as a function of age, the geometric mean IgG1 response was greater in the 6-10 yr old group (1:132) than in the 0-5 yr age group (1:24). Similarly, there was a significant rise in titer in the anti-filarial IgG4 response from 0-5 yr (1:13) to 6-10 yr (1:279). These data confirm our previous observation of elevated IgG levels in the 6-10 yr old population. Preliminary data indicate that these responses as well as anti-filarial IgG2 and IgG3 levels are modulated in 11-15 yr old children. Given the potential association of subclass specific responses with clinical status, longitudinal monitoring of levels of isotype-specific anti-filarial antibody may identify immune responses related to the pathogenesis of filariasis.

- 47 CIRCULATING PARASITE ANTIGEN IN BRUGIA PAHANGI-INFECTED JIRDS. G.J. Weil*, R. Chandrashekar, F. Liftis, C.S. McVay, S.C. Bosshardt, and T.R. Klei. Washington University, St. Louis, MO and Louisiana State University, Baton Rouge, LA.

The purpose of this study was to evaluate parasite antigen detection as a means of noninvasively monitoring Brugia pahangi infection in jirds. A parasite antigen with M_r of 105-110 kDa was identified in sera from i.p. and s.c.-infected jirds by immunoblot with a monoclonal antibody to phosphorylcholine. The same antibody was used in an enzyme immunoassay to measure antigen in jird sera. Parasite antigen was detectable as early as 2 wk after i.p. or s.c. injection of L_3 . Antigen titers increased between 2 and 12 wk and stabilized between 12 and 36 wk after infection in s.c.-infected animals. A different pattern was seen in i.p.-infected jirds with antigen titers peaking at 16 wk and falling significantly between 16 and 32 wk after infection. Parasite antigen titers correlated significantly with adult worm infection intensities in jirds with mature i.p. and s.c.-infections. Antigenemia was also detectable in sera from jirds after i.p. implantation of adult parasites of either sex. However, antigen was not detected in sera from infant offspring of antigenemic, infected mothers. We conclude that parasite antigen detection allows one to monitor B. pahangi development and survival as well as infection intensity in living animals with unprecedented sensitivity and accuracy. This technique should facilitate drug and vaccine studies in this important experimental filariasis model.

SYMPOSIUM: DEVELOPMENTAL FINE STRUCTURE OF ETIOLOGIC AGENTS
IN VECTOR ARTHROPODS
(No abstracts available)

- 48 INTRODUCTION. A.R. Barr. School of Public Health, University of California, Los Angeles, CA.
- 49 INFECTION AND MORPHOGENESIS OF ARBOVIRUSES IN MOSQUITO MESENTERONAL EPITHELIAL CELLS. E.J. Houk. School of Public Health, University of California, Los Angeles, CA.
- 50 AN INSIDE LOOK AT VECTOR COMPETENCE: IN SITU STUDIES OF RIFT VALLEY FEVER VIRUS IN MOSQUITOES. W.S. Romoser. Ohio University, Athens, OH.
- 51 FINE STRUCTURE OF PARASITIC AND SYMBIOTIC ORGANISMS DEVELOPING IN FLEAS. A.F. Hzad and C.B. Beard. University of Maryland School of Medicine, Baltimore, MD, and Yale University, New Haven, CT.
- 52 DEVELOPMENT OF THE LYME DISEASE SPIROCHELE IN IXODES TICKS. A. Spielman. Harvard School of Public Health, Harvard University, Boston, MA.
- 53 DEVELOPMENT OF LEISHMANIA IN PHLEBOTOMINE SANDFLIES. L.L. Walters. Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK.
- 54 DEVELOPMENT OF MALARIA PARASITES IN MOSQUITOES. J.P. Vanderberg. New York University School of Medicine, New York, NY.

PRESENTATION OF THE HOOGSTRAAL MEDAL.

BUSINESS MEETING OF AMERICAN COMMITTEE OF MEDICAL ENTOMOLOGY.

ASTMH POSTER SESSION I

- 55 MONOCLONAL ANTIBODIES REACTIVE WITH SURFACE ANTIGENS ON INFECTIVE LARVAE OF ONCHOCERCA SPP. AND DIROFILARIA IMMITIS DO NOT REACT WITH SURFACE ANTIGENS OF BRUGIA MALAYI OR ACANTHOCEILONEMA VITEAE.

*R.B. Grieve, M. Mika-Grieve, E.W. Cupp, G.R. Frank, M. Mok, D. Abraham and M. Karam. Colorado State University, Fort Collins, CO, University of Arizona, Tucson, AZ, Thomas Jefferson University, Philadelphia, PA and the Onchocerciasis Control Programme, Ouagadougou, Burkina-Faso.

Monoclonal antibodies directed to surface antigens on filarial larvae may be useful in discriminating filarial species and geographic forms within the vector, and in screening DNA libraries for immunogens. Four murine monoclonal antibodies (MAbs) were generated against surface antigens of the infective larvae (L3) of D. immitis and 1 MAb was generated against O. lienalis L3. Screening and cloning of hybridomas was accomplished using intact, fixed homologous L3. L3 and microfilariae of the following filariae were subjected to analyses of stage, species and geographic-form specificities: O. volvulus (forest-form), O. volvulus (savanna-form), O. volvulus (Central American), O. lienalis, B. malayi, A. viteae, and D. immitis. None of the microfilariae were reactive with any of the MAbs. All Onchocerca spp. L3 and D. immitis L3 were reactive with each of the MAbs; however, L3 surface antigens of A. viteae and B. malayi were not reactive with any of the MAbs. None of the MAbs was reactive against phosphocholine, and there was differential reactivity of the MAbs when screened against D. immitis larval metabolic products. These data show evidence of stage and species specificity of larval surface antigens. (Supported by WHO/TDR and The Edna McConnell Clark Foundation)

- 56 STUDIES ON THE REGULATION OF EXPRESSION OF A 16kDa SURFACE - ASSOCIATED ANTIGEN OF BRUGIA MALAYI
Neil Storey* and Mario Philipp. Molecular Parasitology Group, New England Biolabs, 32 Tozer Road, Beverly, MA 01915

We have previously described a 16kDa antigen of *Brugia malayi* which, following infection of the vertebrate host, is expressed by the L3, L4 and adult stages. By virtue of its accessibility to iodination using the Iodogen method, which has been shown to label surface components, we have inferred that it is associated with the parasite surface. The expression of this molecule following infection suggests that it may be functionally important in the vertebrate portion of the parasite's life cycle.

By metabolically labelling parasites with radioactive methionine, and studying the availability of surface - associated molecules to iodination, we have been able to determine when this antigen is expressed and the factors that influence its expression. The antigen was found to be synthesized by pre-parasitic, third - stage larvae, but not expressed at their surface until a point after introduction into the vertebrate host. The relative prominence of this antigen in extracts of metabolically labelled worms, its relative paucity in relation to the total protein content of the parasite and its secretion by the parasite suggest that it may be continually exported and that its association with the surface is probably transient. The results will be discussed in relation to the dynamics of the L3 surface following transition from the intermediate to the definitive host.

ASTMH POSTER SESSION I

- 57 BRUGIA MALAYI: CHARACTERIZATION OF LAMBDA gt11 CLONE EXPRESSING A POTENTIALLY DIAGNOSTIC ANTIGEN. *S. Dissanayake and W.F. Piessens. Harvard School of Public Health, Boston, MA.

In an effort to generate potentially diagnostic/protective antigens of Brugia malayi by recombinant DNA methods, we differentially screened a genomic DNA library of B. malayi in the expression vector lambda gt11 with sera from patients infected with B. malayi or with Wuchereria bancrofti. Clone Bmfs/gt11, which encodes a polypeptide recognized by sera from patients with B. malayi but not by sera from donors with W. bancrofti was selected for further analysis. Conditions for optimal induction, expression, extraction and partial purification of the fusion protein by Sephacryl S300 gel filtration were developed, yielding approximately 1 mg fusion protein/2 liter bacterial culture.

Clone Bmfs/gt11 contains a DNA insert of approximately 250 bp. The nucleic acid sequence of 160 bp of the insert has been determined so far by subcloning a KpnI-SacI subfragment into M13mpl8. The 5' end of the insert is in continuous reading frame with the gt11 LacZ gene. This M13 subclone, when used as a probe, detects two major bands on Southern blots of genomic DNA from B. malayi partially digested with EcoRI and hybridizes to poly A-mRNA from microfilariae. Expression of this apparently B. malayi-specific polypeptide in an expression system yielding soluble recombinant proteins could be a potential source of a B. malayi-specific diagnostic antigen.

- 58 BRUGIA MALAYI: ANTIBODIES TO AN ONCHOCERCA VOLVULUS HEAT SHOCK PROTEIN 70 IN AMICROFILAREMIC JIRDS. J. Yates*, K. Schmitz, N. Rothstein, and T.V. Rajan, Oakland University, Dept. of Biological Sciences, Rochester, MI and University of Connecticut Health Center, Farmington, CT.

We have previously observed that amicrofilaremic people in a Wuchereria endemic area, with or without signs of filariasis, differentially produced antibodies to a heat shock 70 fusion protein. In the present study western blot assays were used to determine if experimentally immunized inbred jirds would also produce antibodies to this protein. Jirds were immunized by chemotherapeutic abbreviation of three subcutaneous infections, each of 2 weeks duration. The experimental macrofilaricidal compound CGP 20376, kindly supplied to us by the Cieba-Geigy Company, was used to terminate the immunizing infections. Eleven of 12 jirds immunized in this study remained amicrofilaremic 6 months after a subcutaneous challenge infection with 100 B. malayi L3. Sera from nine of these amicrofilaremic jirds contained antibodies to the heat shock fusion protein while the 12 microfilaremic control jirds failed to recognize it. Reactivity of these sera with other filarial fusion proteins is currently being evaluated and the usefulness of this fusion protein as a potential protective antigen in jirds is under study.

ASTMH POSTER SESSION I

- 59 VARIATIONS IN COMPLEMENT MEDIATED CELLULAR ADHERENCE AND CYTOTOXICITY TO MICROFILARIAE OF BRUGIA PATEI, BRUGIA MALAYI AND BRUGIA PAHANGI.
U.R. Rao, *B.H. Kwa, and A.C. Vickery.

College of Public Health, University of South Florida, Tampa, Florida.

The complement of fresh serum from normal heterozygotic Balb/c (nu/+) and nude (nu/nu) mice was activated by sheathed microfilariae (mf) of B. pahangi and B. malayi but not by B. patei. Mouse C3 was detected on the sheath of B. malayi and B. pahangi but not on B. patei mf by immunofluorescence. Bound complement promoted macrophage and neutrophil mediated adherence and cytotoxicity to mf. However, macrophages and neutrophils from nu/+ and nu/nu adhered to exsheathed mf of B. patei and killed them in the presence of fresh normal serum. C3 molecules were detected on the surface of exsheathed B. patei and B. malayi mf. Fresh normal mouse serum (nu/+ and nu/nu) depleted of complement by heating at 50° C for 20 min or at 56° C 30 min and by anti-mouse C3, failed to promote cell adherence to these parasites. EDTA but not EGTA abolished the adherence activity to sheathed and exsheathed mf of B. malayi, whereas both EGTA and EDTA abolished the adherence activity to exsheathed mf of B. patei and sheathed mf of B. pahangi, suggesting different pathways of complement activation by these closely related species. Macrophages and neutrophils from peritoneal exudates of nu/+ and nu/nu were equally potent in adherence and cytotoxicity to mf. Mf pretreated with N-acetyl glucosaminidase and L-fucosidase augmented the adherence and cytotoxicity of macrophages to sheathed mf of B. patei, B. malayi and B. pahangi suggesting the possible involvement of exposed surface carbohydrates in enhanced cellular cytotoxicity. These results strongly indicate differences in surface complexities between the species in activating complement, cellular adherence and cytotoxicity.

- 60 ULTRASTRUCTURAL CHANGES IN THE LYMPH NODES OF BRUGIA MALAYI INFECTED NUDE MICE PRIOR TO THE ONSET OF LYMPHATIC DILATION. E. Toro, B.H. Kwa, *A.C. Vickery. College of Medicine and College of Public Health, University of South Florida, Tampa, Florida.

Transmission electron microscopy was used to study parasitized lymph nodes and associated afferent lymphatics of male C3H/HeN nude mice infected with Brugia malayi, 6-9 months post-inoculation, before the onset of lymphatic dilation. No congestion of the lymphatics was observed in contrast to the severe blockage by numerous thrombi, in the lymphatics of immunologically reconstituted nude mice. Heavy collagen deposition was, however, observed in the interstitial spaces of the paracortex of lymph nodes from parasitized mice; collagen deposition occurred to a degree not observed in control nodes. Furthermore, the collagen fibrils were randomly diffused in the interstitia quite unlike the discrete uniformly-organized bundles seen in the nodes of unparasitized animals. Aspirated lymph from parasitized and dilated lymphatics contained extremely high protein concentrations which would result in elevated osmotic pressures. Furthermore, endothelial cell cultures from parasitized lymphatics could be stimulated to grow in vitro in the presence of B. malayi excretory-secretory antigens. Therefore, we propose that lymphangiectasis, seen in long-term parasitized nude mice, might be the result of increased osmotic pressure due to free collagen, accumulation of intraluminal tissue fluid, and endothelial hyperplasia to accommodate the enlarged luminal volume. Thymus dependent immune responses apparently are not involved. (Supported by UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases ID 870382)

ASTMH POSTER SESSION I

61 HUMAN IMMUNE RESPONSES TO *Onchocerca volvulus*: POSSIBLE RELATIONSHIP BETWEEN SPECIFIC ISOTYPIC ACTIVITIES TO ANTIGENS AND STATE OF IMMUNITY.

*A.E. Boyer, V.C.W. Tsang, M.L. Eberhard, J.A. Brand, W. Zhou, and L. Laughnan. Division of Parasitic Diseases, Centers for Infectious Disease, Centers for Disease Control, Atlanta, GA. 30333.

Onchocerciasis, a leading cause of human blindness, affects an estimated 20-50 million persons. Blindness turn productive individuals into burdens for their families and community. The fact that certain long term residents in endemic areas of Guatemala never develop the disease, suggests the existence of some mechanism which confers immunity. Humoral immune response in infected and "immune" individuals with equivalent exposures to *O. volvulus* were assayed by FAST-ELISA and Immunoblot. Aqueous soluble and particulate antigens were derived from human nodules. Immunoblots of sera from infected individuals show only IgG1 and IgG4 isotypes reactions to crude *O. volvulus* nodular antigens. In infected persons, specific serum IgG1 levels to these antigens (FAST-ELISA) are 5 to 10 fold higher than all other isotypes, and 5 to 10 fold higher than the IgG1 levels of immune individuals and normal controls. Serum samples of immune individuals show no reaction of any isotype on immunoblot, but show elevated levels of IgG3 when quantitated by FAST-ELISA, suggesting that the antigens which are IgG3-specific may well be labile to SDS treatment. In many cases, the specific reactivity ratios of IgG3:IgG1 in immune individuals were as high as 3:1. In "healthy" controls, the ratio of total IgG3:IgG1 is 1:10. When compared to levels in immune individuals this is significant and suggests a possible mechanism that confers immunity.

62 IMMUNOLOGICAL ACTIVATION ASSOCIATED WITH IVERMECTIN-INDUCED MICROFILARIAL CLEARANCE. P. Lammie*, M. Eberhard, R. Bryan, F. Richards, D. McNeeley, and H. Spencer. ICIDR Program, Tulane University, New Orleans, LA; CDC, Atlanta, GA; and Ste. Croix Hospital, Leogane Haiti.

The presence of circulating microfilariae has been associated with alterations in B and T cell function. Studies with diethylcarbamazine (DEC) have demonstrated a restoration in filarial antigen specific responsiveness of PBMC from DEC-treated subjects. In this study, the influence of ivermectin, a microfilaricidal drug, on immunologic parameters was evaluated. Thirty microfilaremic Haitian subjects were treated with a single 20 ug/kg dose of ivermectin. Post-treatment microfilaremias were reduced to 0.3/ml from a pre-treatment median microfilarial density of 554/ml. PBMC were collected at pre- and 120 hr post-treatment time points and cryopreserved. Paired samples were analyzed in parallel in *in vitro* blastogenesis assays. Mean proliferative responses of post-treatment PBMC to PHA and PPD, as well as to crude extracts of *Brugia pahangi* and *Ascaris* were elevated relative to pre-treatment values, significantly so in the case of the nematode extracts. These changes are due to a significant increase in background proliferative reactivity following treatment ($P < 0.02$). No change in the mean stimulation ratio to *B. pahangi* was observed, although the number of individuals defined as responders increased from 13 to 17 of 30 following treatment. Commensurate with increased background proliferation, an increase in spontaneous production of specific anti-filarial antibody was observed in studies with a subset of patients. These changes represent the host response to release of circulating antigens and are associated with clinical manifestations. (Supported in part by WHO Technical Service Agreement 05-181-41, NIH grant AI-16135, and Merck Sharpe, and Dohme)

ASTMH POSTER SESSION I

- 63 **ISOLATION OF A HYPERVARIABLE REPETITIVE DNA ELEMENT (VNTR) FROM THE GENOME OF THE HUMAN FILARIAL PARASITE, *WUCHERERIA BANCROFTI*.** S. Natarajan* and T. V. Rajan, Department of Pathology, University of Connecticut Health Centre, Farmington, CT.

Repetitive DNA exists in 3 different distributions in the eucaryotic genome: tandem repeats where monomers are present in a contiguous array as a single unit; interspersed repeats in which the individual members are distributed as single copies widely over the entire genome; and a recently reported class called Hypervariable Repeats or Variable Number of Tandem Repeats (VNTR) where the members are interspersed throughout the genome, with each member containing a variable number of short monomers in a tandem arrangement. These VNTR's are a powerful tool for the identification and differentiation of closely related organisms and subspecies. We had earlier reported the cloning and characterization of a VNTR family in the genome of *Brugia malayi*. In order to study the evolution of VNTR's in the filarids, we screened a *Wuchereria bancrofti* genomic DNA library with one member of the *Brugia* hypervariable repeat family as probe and isolated the *Wuchereria* homologue. This is of particular interest as *W. bancrofti* is the parasite responsible for most cases of human lymphatic filariasis and various physiological differences have been described amongst different isolates. This hypervariable repeat should enable us to investigate genome structure and evolutionary relationships among the various isolates.

- 64 **A FILARIAL ANTIGEN RECOGNIZED BY MANY PATIENTS WITH HUMAN LYMPHATIC FILARIASIS BEARS STRONG SEQUENCE HOMOLOGY TO MAMMALIAN TYPE 4 (BASEMENT MEMBRANE) COLLAGEN**

C. R. Vanamala~*, C. Werner#, and T. V. Rajan~.
~Department of Pathology, University of Connecticut Health Centre, Farmington, CT 06032 and #Department of Microbiology & Immunology, Albert Einstein College of Medicine, Bronx, N.Y.

We have previously cloned two genomic *Brugia malayi* fragments that encode antigens recognized by sera from many individuals from a filariasis endemic area, including endemic normals. One clone encodes a protein which appears to evoke primarily antibodies of the IgE isotype. We used the insert in this clone to screen *B. malayi* genomic libraries in order to isolate larger fragments and determine the precise identity of the antigen. We have now obtained two overlapping genomic clones that, together, contain over 8 kb. of *B. malayi* genomic DNA. Sequence analysis of these clones indicates that coding sequence is widely dispersed over the entire 8 kb. The sequence available to date reveals that the protein encoded by this gene bears strong homology to mammalian type IV collagen, a constituent of basement membranes.

ASTMH POSTER SESSION I

65 ISOLATION AND CHARACTERIZATION OF *WUCHERERIA BANCROFTI* RECOMBINANT CLONES RECOGNIZED BY ANTISERA TO A POTENTIALLY PROTECTIVE 43 KD ANTIGEN OF *BRUGIA MALAYI* INFECTIVE LARVAE.

*D. O. Freedman, N. Raghavan, T. B. Nutman and E. A. Ottesen. Laboratory of Parasitic Diseases, NIAID, Bethesda, MD.

Infection-free, putatively immune individuals resident in an area endemic for *W. bancrofti* have been reported to recognize selectively a 43 kD infective larval stage (L₃) antigen of *B. malayi*. For further characterization of this potentially protective antigen, rabbit antiserum was raised against this 43 kD antigen isolated from *B. malayi* L₃ preparations run on 2-dimensional gels, and this serum was used to isolate recombinant clones from a *W. bancrofti* genomic expression library in λ gt11. Of the 150,000 recombinants screened, 16 positives were plaque purified based on strong antibody reactivity. When recombinant antigen extracts from 7/16 clones were analyzed by immunoblotting with anti β -galactosidase and affinity purified anti-43 kD antibodies, 5 clones with fusion products of M_r 118 kD, 120 kD and 150/200 kD were obtained. The study of these recombinant clones should provide insight into the molecular nature of the 43 kD antigen and its possible role in protective immunity against lymphatic filariasis.

66 MAPPING THE B CELL EPITOPES OF FILARIAL PARAMYOSIN WITH SERA FROM PATIENTS WITH ONCHOCERCIASIS. *C. Steel, R. Limberger, T.B. Nutman, C. Maina, L.A. McReynolds. Laboratory of Parasitic Diseases, NIH, Bethesda, MD, and New England Biolabs, Beverly, MA.

Paramyosin has been identified as an immunogenic molecule in several filarial species and also has been implicated as a potential vaccine candidate for schistosomiasis. A cDNA clone (2.55 Kb) coding for *Dirofilaria immitis* paramyosin has been sequenced and shows one long open reading frame with no prolines. The sequence has a 99% protein homology with *O. volvulus* (Ov) paramyosin and a 92% and 33% homology with the paramyosin of *C. elegans* and *S. mansoni* respectively. Paramyosin was expressed in a vector containing the maltose binding protein (MBP) and purified using amylose column affinity chromatography. Antigenicity of the cloned antigen was demonstrated by ELISA using sera from Guatemalan patients infected with Ov. To map the antigenic sites of the molecule, six subclones of paramyosin spanning all but 150 nucleotides were fused to MBP and sera from 18 Ov infected patients were tested by ELISA with these six fragments. Though the regions of the protein recognized and the intensity of recognition varied among patients, the primary immunogenic B cell epitope was identified at the amino terminal end of the molecule with a secondary area of reactivity lying just 5' to nucleotide 1990. This approach should allow for the identification of defined epitopes important in the induction of antibody responses in filarial infections.

ASTMH POSTER SESSION I

- 67 Susceptibility of immunodeficient mouse strains to infection with larvae of *Brugia malayi*. Carole J. Prain and Mario Philipp. Molecular Parasitology Group, New England Biolabs, 32 Tozer Road, Beverly, MA 01915.

The immune effector mechanisms capable of killing invading larvae of the human parasite *Brugia malayi* are unknown. We are trying to identify mechanisms that enable mice to resist *Brugia* infections in order to gain insight as to the type of immune response to which these parasites are susceptible. For this purpose we have examined the course of primary and secondary *B. m.* infections in several mouse strains which possess genetically defined immune defects, and compared them, where possible, to histocompatible normal mice. Strains so far examined include CBA/N mice which possess an X-linked genetic defect resulting in impaired IgM and IgG3 antibody responses to certain antigens and SJL mice which exhibit lowered IgE responses and natural killer cell functions. Worm recoveries in the CBA/N mouse strain were significantly higher than seen in the CBA/Ca control mice up to day 21 of a primary infection. However, challenge infections given in CBA/N and CBA/Ca mice resulted in reduced worm burdens evident by day 7, with no significant difference between the strains. The BALB/c strain was used as normal, control mice and compared with the SJL strain. Worm numbers in BALB/c mice showed a steady decline from day 7 of a primary infection onwards with very few or no parasites recovered by day 21. During secondary infections, BALB/c mice showed a substantial reduction in worm numbers by day 7. The number of worms recovered from SJL mice did not alter significantly until day 21 of a primary infection, after which they declined to levels similar to those recovered from the BALB/c mice at the same time point. During secondary infections, significantly more worms were recovered from SJL compared to BALB/c mice up to day 21. These results suggest that antibodies of IgM, IgG3 and IgE isotypes, in addition to natural killer cells may play important roles in murine immunity to *Brugia* infections.

- 68 AN *ONCHOCERCA VOLVULUS* ANTIGEN WHICH IS PREFERENTIALLY RECOGNIZED BY SERA FROM 'PUTATIVELY IMMUNE' ENDEMICIS.

* S.E. Roemer, T.B. Nutman, M.W. Southworth, F.B. Perler, New England Biolabs, Inc., Beverly, MA and Laboratory of Parasitic Diseases, NIH, Bethesda, MD.

Onchocerca volvulus is a leading cause of infectious blindness. The range of human responses to the parasite include symptomatic and asymptomatic infected individuals, and 'putatively immune' endemics (Ward et al., 1988). In order to study differences in antibody responses by these defined groups, OV λ gt11 cDNA libraries were screened with pooled sera. Two clones, OI5 and OI3, were selected using pooled sera from the 'putatively immune' group. All individual sera from that pool recognized both cloned antigens in Western blot analysis, whereas only 11% and 26% of infection sera recognized OI5 and OI3 antigens, respectively. Restriction mapping and DNA sequencing indicate that OI5 and OI3 encode portions of the same gene. OI3 (1.4kb) encodes approximately a 45kDal peptide which has been overexpressed as a fusion protein with the *E. coli* maltose binding protein (NE Biolabs MBP vector system). OI3 is very lysine rich at its carboxy terminal end, and no homology has been detected to previously published protein sequences. OI5 (0.7kb) begins 66 nucleotides 5' to the beginning of OI3 and does not contain the lysine rich region found in OI3. Milligram amounts of OI3 fusion protein have been purified and used to generate specific anti-OI3 serum. Synthesis of large amounts of recombinant OI5 and OI3 antigens will enable us to further examine the preferential recognition of this protein by 'putatively immune' individuals. Ward et al. (1988) J. Infect. Dis. 157, 536-543.

69 **ELEVATED TISSUE EOSINOPHILIA AND SERUM IgG IN EXPERIMENTAL MURINE OCULAR ONCHOCERCIASIS**

Eric R. James, Amy Hodgson-Smith, Martha Jackson-Gegan, Brad Smith, David McLean, Heather L. Callahan and John Renfro III, Department of Ophthalmology, Medical University of South Carolina, Charleston, SC

We are attempting to define a mouse model for ocular onchocerciasis. Mice received two injections of 2×10^4 microfilariae (mf) followed by 1×10^5 mf, or 25 injections of 2×10^4 mf of *Onchocerca cervicalis* subcutaneously immediately below the left eye. Mf could be detected in ocular tissues from 24 h post injection (PI) as determined by slit lamp examination and LM histology. Circulating eosinophils were transiently elevated to a maximum of 4% (in C57BL/6 mice) at 7 d PI. Circulating neutrophils were lower with this strain (<10%) and elevated (>30%) in C3H mice. Tissue eosinophilia was calculated from counts of individual cells in the limbal sclera and in C57BL/6 mice reached approximately 15% by 7 d PI in the first infection of 2×10^4 mf and 30% at 3 d after the 1×10^5 mf infection, declining gradually to a baseline of <3% by 28 d PI. Infected C57BL/6 mice treated with diethylcarbamazine (DEC) showed no change in circulating eosinophil numbers but a marginal additional rise in scleral tissue eosinophilia to 38%. In ivermectin-treated infected animals and in non-infected DEC-treated control mice there was no significant further increase in circulating or tissue eosinophilia. Tissue eosinophilia was marginally lower in Balb/C and C3H mice, while numerous mast cells were observed in C3H mice. ELISA indicated that IgG levels were markedly elevated in infected, DEC-treated and ivermectin-treated groups in all 3 strains of mice and particularly in the C57BL/6 strain. There was only a marginal elevation of IgM and IgA titers. IgG titers peaked earlier and remained elevated longer in the ivermectin-treated mice compared to the DEC-treated mice. All IgG isotypes tested (1, 2a, 2b and 3) were elevated. Overall, C57BL/6 mice responded more aggressively immunologically to *O. cervicalis* infection and this may correlate with clinical ocular changes seen with this strain (reported previously) suggesting that C57BL/6 strain mice may be a useful small animal model for chronic onchocerciasis. Supported by EY 06462, EY 07542 and the Edna McConnell Clark Foundation.

70 **IDENTIFICATION OF RODENT HOSTS FOR LARVAL STAGES OF *ONCHOCERCA LIENALIS***. D. Abraham*, J.B. Lok, A.J. Ripepi and A.M. Lange. Thomas Jefferson University, Philadelphia, PA and University of Pennsylvania, Philadelphia, PA.

Research on the biology and immunology of infections with *Onchocerca* spp. has been hindered by a lack of suitable laboratory-animal hosts. The objective of this project was to screen a variety of inbred rodents to determine their susceptibility to infection with third-stage larvae (L-3) of *O. lienalis*. In the primary screen, 5 strains of mice (C57BL/6, DBA/2, SWR, SJL and CBA), 5 strains of rats (Buffalo, Lewis, Copenhagen 2331, Brown Norway and Wistar Furth), birds (*Meriones unguiculatus*) and multimammate rats (*Mastomys natalensis*) were tested. Animals were infected by implantation of L-3 subcutaneously, in diffusion chambers covered with 5.0µ pore-size membranes. These membranes allowed unimpeded host cell entry into the chambers. After 7 days the chambers were recovered and larval viability and growth assessed. Approximately 45% of inoculated larvae were recovered live regardless of host tested. The recovered larvae were approximately 25% longer than the inoculated L-3. Larvae were implanted in CBA and DBA mice in chambers covered with membranes which prevented host cells from entering; survival and growth rates of the larvae were not altered by the absence of cells from the chambers. Cryopreserved larvae were implanted in chambers with 5.0µ membranes in CBA and DBA mice and Wistar Furth rats for 3, 7, 14, 21 and 28 days. A maximum of 50% of the larvae implanted were recovered on Day 3 and up to 33% on Day 28. Larval lengths increased by as much as 35% of L-3 length after 28 days *in vivo*. These experiments demonstrate that inbred strains of mice and rats are susceptible to infection with larval *O. lienalis*. Furthermore, the results suggest that these rodent-models will be valuable for the study of the immunobiology of *Onchocerca* spp. infections.

ASTMH POSTER SESSION I

- 71 LARGE SCALE IN VITRO PRODUCTION OF ONCHOCERCA VOLVULUS
FOURTH STAGE LARVAE. Brotman, B., Smith, A.B., Prince, A.M.
 The Lindsley F. Kimball Research Institute of the New York
 Blood Center, New York, NY and Vilab II, The Liberian
 Institute for Biomedical Research, Robertsfield, Liberia.
 To provide large quantities of L4 larvae for immunologic and
 biochemical studies, we have optimized methods for in vitro
 rearing from the third larval stage (L3). The availability of a
 large number of freshly dissected L3's (from 6-13,000/month)
 permitted us to methodically examine many aspects of this
 technology. To reduce microbial contamination L3's were passed
 through 20% Percoll. Eighty-eight percent (+/-13%) of larvae were
 retrieved after this procedure. These were plated individually
 to 96 well plates in medium containing equal volumes of IMDM and
 NCTC-135, supplemented with 20% fetal calf serum and 2X Cefoxitin,
 Streptomycin and Fungizone (held at 37°C in 5% CO₂). Bacterial
 contaminants (the majority of which were nonfermentative gram (-)
 bacilli) found in vitro were most frequently sensitive to
 doxyryline, tetracycline, cefoxitin and chloramphenicol, of
 limited sensitivity to gentamicin and resistant to penicillin and
 ampicillin. Tetracycline and chloramphenicol greatly inhibited
 molting. Contamination occurred in less than 1% (49/6469) of L3's
 plated using the above cleaning and antibiotic regimen. The pH
 of dissecting medium did not seem to alter rates of viability as
 L3's held at pH 6, 7 and 8 for 4 hours prior to cultivation had
 similar rates of exsheathment. Likewise L3's dissected from the
 head, thorax and abdomen of black flies molted with equal
 efficiency. Larvae could be held up to 24 hours at 4°C prior to
 cultivation. Rates of molting were compared using a feeder layer
 of monkey kidney (MK-2) cells, conditioned medium (CM) from
 confluent MK-2 cells and with the addition of normal chimpanzee
 monocytes. Rates of molting were 54% (+/-8%), 50% (+/-9%) and 59%
 (+/-8%), respectively. Up to 80% of molting occurred between days
 3-5 in vitro with slightly delayed molting (between days 5 and 7)
 seen when CM was used.

- 72 BRUGIA PAHANGI IN DOGS: BASIC PARASITOLOGICAL DATA. J. W. McCall,*
 M. D. Dzimianski, J. J. Jun, and P. Supakorndej. Department of
 Parasitology, College of Veterinary Medicine, University of Georgia,
 Athens, GA 30602.

The dog has been used as an experimental host for B. pahangi for a
 number of years, but little basic parasitological data is available. In
 view of this, 36 beagles were each given 200 third-stage, infective larvae
 (L3) by SC inoculation in the dorsum of the right hind paw. Microfilarial
 (MF) counts were made at 81, 89, 95, 102, 108, 112, 116, 123, 137, 151, and
 154 days PI. At day 81, 32 of the 36 dogs had patent infections. By day
 102, all of the 36 dogs had been positive for MF on at least 1 occasion.
 At that time, 2 amicrofilaremic dogs and 2 dogs with only 1 MF/ml were set
 aside for necropsy at a later date, and the remaining 32 dogs were randomly
 allocated to 2 control groups and 6 treatment groups of 4 dogs each. The
 data on the treated dogs will be presented later. MF counts in the 8
 control dogs were followed until necropsy, which began at 165 days PI. The
 average MF count in these 8 dogs increased from 17.5/ml on day 102 to
 236.4/ml on day 157. Ten selected lymphatics, the testicles of males, and
 the heart and lungs were examined for worms. An average of 36.1 worms per
 dog were recovered from the 8 dogs, with most (97.3%) of the worms from the
 right hind limb (86.9%), abdominal lymph nodes (7.3%), and the right
 inguinal lymph node (3.1%). No worms were recovered from the 4 dogs set
 aside earlier. In conclusion, by SC inoculation of L3 in the dorsum of a
 hind paw, which allows for a limited necropsy, B. pahangi infections in
 dogs are sufficiently uniform, manageable, and predictable for routine use,
 particularly as a tertiary screen in a filaricidal drug development program.

ASTMH POSTER SESSION I

- 73 GRANULOMATOUS REACTIVITY OF FRACTIONATED BRUGIA PAHANGI ADULT ANTIGENS IN HOMOLOGOUSLY INFECTED JIRDS. R.G. Farrar, T.R. Klei*, M.D. West, R.C. Montelaro, C.S. McVay and S.U. Coleman. Louisiana State University, Baton Rouge, LA.

Soluble somatic extracts of adult Brugia pahangi were fractionated by sequential lentil lectin affinity, and reverse phase HPLC. The resulting glycoprotein fraction and HPLC fractions were assayed for their ability to induce granulomas when coupled to sepharose beads that were subsequently embolized in lungs of jirds with infections of 60 or 150 days duration (DPI). Fractions were also tested in blastogenic assays using lymphnode (LN) and spleen cells from jirds with similar infections. Granulomas induced by unfractionated B. pahangi antigen and eluate from the lentil lectin column produced similar reactions which were maximal at 60 DPI and significantly reduced at 150 DPI. Three fractions (B,C,D) of flowthrough from the lentil lectin column induced little or no reaction at either time. Reaction to one fraction (E) was significantly > any other fraction at 60 DPI. The remaining fractions (F,G) were similar in granulomatous reactivity to starting fractions. Fraction coated bead induced granuloma formation correlated with the intensity of pulmonary perivascular eosinophil infiltrate. Blastogenic responses of LN cells and splenocytes to crude Ag were similar to previous reports. Splenocyte reactivity corresponded to granulomatous reactivity to crude Ag. Fractions B, C, and D appear to be suppressive in cell cultures at all concentrations tested. Correlations of in vivo granulomatous reactivity and in vitro blastogenic responses were not demonstrable with other fractions. These studies indicate that in vivo pulmonary granuloma formation is useful in identification of parasite proteins potentially important in the induction of filarial induced inflammatory responses. Supported by NIH grant AI-19919.

- 74 CHARACTERIZATION OF A GENOMIC CLONE FROM ONCHOCERCA VOLVULUS HOMOLOGOUS TO A BRUGIA MALAYI GENE FOR A POTENTIALLY PROTECTIVE ANTIGEN. Michael A. Kron*, Klaus Erttmann, Thomas Unnasch and Bruce Greene. Case Western Reserve University, Cleveland, Ohio, and University of Birmingham, Birmingham, Alabama.

In the absence of a suitable animal model for the human filarial parasite, Onchocerca volvulus, direct identification of immunologically significant antigens is difficult. Recently, Nilsen and colleagues have reported the isolation of a clone encoding a potentially protective antigen from Brugia malayi. Southern blot analysis of O. volvulus genomic DNA using the B. malayi cDNA as a probe suggested that a homologue exists in O. volvulus. An O. volvulus EMBL3 genomic library was screened with the Brugia cDNA and a probable full length clone, OVB2, was isolated. Southern analysis determined that probable coding regions within OVB2 were distributed over 4 kilobases, and thus far, DNA sequence data was generated on 3 kilobases of OVB2. Analysis of sequence data demonstrated a 75-85% DNA homology between open reading frames in OVB2 and the Brugia cDNA. Northern blot analysis of mRNA from adult female O. volvulus probed with ³²P labelled OVB2 demonstrates no definite signal. Evidence indirectly supportive of the rarity of this message includes absence of cDNA clones in lambda GT11 expression libraries, and failure to amplify a cDNA using gene specific primers in the polymerase chain reaction. These data suggest that a homologue of a protective B. malayi gene exists within the genome of O. volvulus, but that in contrast to Brugia, the gene is not highly expressed in adult female O. volvulus. This work demonstrates an alternative strategy for identification of potentially significant antigens in O. volvulus.

ASTMH POSTER SESSION I

- 75 PHOSPHOCHOLINE CONTAINING ANTIGENS OF BRUGIA MALAYI
NON-SPECIFICITY SUPPRESS LYMPHOCYTE FUNCTION. R.B.
LAL*, V. KUMARASWAMI⁺, C. STEEL**, T.B. NUTMAN**. Div. of
Trop. Pub. Hlth., USUHS, Bethesda, MD; Lab. Parasitic Dis., NIH, Bethesda,
MD and Tuberculosis Research Institute, Madras, India.

The immunosuppressive effect of Brugia malayi antigen (BmA) on PHA driven T cell proliferation was evaluated in Patients with filariasis (n=14) and compared to "control" individuals (n=12). When peripheral blood lymphocytes were co-cultured with BmA and PHA, BmA markedly suppressed the T cell proliferative response to PHA in both filarial patients and control individuals in a dose-dependent manner. The suppression resulted neither from any direct toxicity of BmA nor from non-specific absorption of the PHA-mitogenic activity by BmA.

The major suppressive component appears to be phosphocholine (PC), an immunodominant-molecule present in abundance on filarial parasites and on circulating filarial antigen. Both purified PC as well as PC containing antigens affinity purified from BmA were capable of suppressing the proliferative responses of co-cultured autologous lymphocytes to PHA. The suppressive activity was not abolished by mitomycin-c treatment and was greater in patients with filariasis than in normal controls, suggesting that previous sensitization to PC containing antigens determines the magnitude of the suppressive effect of PC-antigen. Further, as induction of the suppressive activity was completely abrogated when antigen pre-treated cells were T-cell depleted, the suppressive effect appears to be mediated primarily by T cells.

- 76 ACTIVATION OF JIRD B AND T LYMPHOCYTES BY RECOMBINANT
FILARIAL ANTIGENS N.J. Pieniazek*, D.L. Ellenberger, and
P.J. Lammie. Parasitic Diseases Branch, CDC, Atlanta, GA;
Dept. of Microbiology, LSU Medical Center, New Orleans, LA, and
Emory University, Atlanta, GA.

Patent Brugia pahangi infections are associated with alterations in in vitro and in vivo filarial antigen specific responsiveness. Understanding the basis of these immunoregulatory changes has been hampered by the complexity of parasite antigens. Recombinant B. pahangi antigens provide defined tools to analyze immunoregulatory processes. In preliminary experiments, jirds were immunized with bacterial lysates of B. pahangi clone 1112, KL or with control lysates containing no insert. Sera from animals immunized with the B. pahangi derived clones, but not the control animals recognized extracts of B. pahangi by ELISA. In in vitro blastogenesis assays, lymph node cells from KL- and 1112- immunized jirds, but not control jirds responded to B. pahangi antigen. Similarly, spleen cells from KL- and 1112- immunized animals, but not control animals produced significant quantities of B. pahangi specific antibody in vitro. These antigens should provide useful probes to dissect immunoregulatory mechanisms in B. pahangi infected jirds. (Supported in part by NIH grant AI-24459)

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- 77 INTRACYTOPLASMIC CIRCUMSPOROZOITE PROTEIN IS TRANSLOCATED TO THE SURFACE OF GLIDING MALARIA SPOROZOITES AT THEIR ANTERIOR ENDS.
Michael J. Stewart* and Jerome P. Vanderberg. Department of Medical & Molecular Parasitology, New York University School of Medicine, New York, NY 10016.

As they glide, Plasmodium sporozoites leave trails of circumsporozoite (CS) protein that trace their patterns of movement. The length of these trails reveals quantities of CS protein that cannot be accounted for solely by release of pre-existing surface protein, but is indicative of a continuous introduction of new protein to the surface from an intracytoplasmic pool. We have previously suggested that intracytoplasmic CS protein is first translocated to the surface of gliding sporozoites at their anterior (leading) ends and then flows posteriorly along the surface during sporozoite movement. This mechanism might explain the circumsporozoite precipitation (CSP) reaction.

By videomicroscopic observations of P. berghei sporozoites, we now show that sporozoites immobilized with cytochalasin b do not generate trailing CSP reactions upon exposure to anti-sporozoite antibodies; instead, CSP reaction-like precipitates are formed at their anterior ends. In addition, the release of CS protein by sporozoites into the medium is unhindered by cytochalasin treatment. Finally, motile sporozoites translocate latex beads along their surface, whereas cytochalasin-immobilized sporozoites do not. The data suggest that Plasmodium sporozoite motility is associated with a cytochalasin-insensitive translocation of CS protein to the sporozoite surface, coupled with a cytochalasin-sensitive flow of CS protein posteriorly along the surface.

- 78 DETECTION OF THE CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM FALCIPARUM IN MOSQUITO TISSUES.
Claudia F. Golenda, Imogene Schneider, and Robert A. Wirtz, Department of Entomology, Walter Reed Army Institute of Research, Washington DC. 20307-5100

Although the circumsporozoite protein (CS) of Plasmodium species has been the primary antigen used in the development of malarial vaccines, little is known about its physiological role. It has been suggested that the CS protein is important in motility since sporozoites leave behind trails of CS protein during gliding motility. The distribution of the CS protein of Plasmodium falciparum was monitored in Anopheles stephensi mosquitoes using an immunohistochemical assay that utilized alkaline phosphatase directly conjugated to a monoclonal antibody to the CS protein of P. falciparum. Sites of phosphatase activity were permanently stained red using new fuchsin dye and cell structure defined by hematoxylin counterstaining. The CS protein first was detected in mosquitoes fixed 8-10 days after an infective blood meal where it was associated with the peripheral vacuoles of immature oocysts. As oocysts matured, detectable CS protein increased. Sporozoites detected in the hemolymph were highly immunogenic as were remanent oocysts. Although penetration of the salivary glands by sporozoites was not observed, congregation of parasites in the vicinity of the gland and adhesion to the gland were seen. The CS protein was detected in small circular structures (1-2 microns in diameter) on the surface of the salivary glands. While the CS protein was readily detected on the surfaces of both salivary glands and sporozoites located outside the glands, it was difficult to detect it on sporozoites within the lumen of gland cells. When detected, it appeared as a diffuse material around sporozoites, often associated with the periphery of vesicular structures containing bundles of parasites. Detection of the CS protein in suspensions containing sporozoites and gland debris indicated that isolated sporozoites labeled differently from parasite bundles and that there was CS protein associated with the gland debris. The association of CS protein with the substratum with which the parasite has made contact appears to support the hypothesis of its importance in motility.

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- 79 A RECOMBINANT *P. FALCIPARUM* CIRCUMSPOROZOITE (CS) PROTEIN DESIGNED WITH A HYDROPHOBIC DECAPEPTIDE ANCHOR (R32FT) INCREASES IMMUNOGENICITY IN CS-REPEAT RESPONDER AND NON-RESPONDER MICE WHEN HYDROPHOBICALLY COMPLEXED TO PROTEOSOMES.

R. Jaffe*, L. Loomis, W. Zollinger, D. Monheit, W. Ballou, A. Fisher, M. Gross, J. Young, J. Sadoff, and G. Lowell, Walter Reed Army Institute of Research, Washington, DC and Smith Kline and French Laboratories, Philadelphia, PA.

We have previously shown that adding a hydrophobic amino acid anchor containing cysteine to the terminal end of a small peptide, increases immunogenicity in mice. In addition, the immunogenicity of such anchored peptides are greatly enhanced by hydrophobically complexing them to meningococcal outer membrane proteins (proteosomes). A hydrophobic anchor was added to R32LR, a recombinant product with 32 tandem tetrapeptide repeats derived from the *P. falciparum* CS protein, by adding a synthetic oligonucleotide encoding for a series of 10 hydrophobic amino acids to the 3' end of the gene. This protein, R32Ft, was very well expressed in *E. coli* and was purified using a modification of the procedure used to purify R32LR.

In contrast to R32LR, R32Ft was immunogenic without adjuvants even in H-2d mice (BALB/c) which are genetically restricted from responding to the CS-repeat epitope. Complexing R32Ft to proteosomes markedly enhanced its immunogenicity, without any other adjuvants, in all mouse strains tested. Outbred CD-1 mice, immunized with R32FT complexed to proteosomes, gave the highest titer: 102,400 units. BALB/C mice and C57BL/6 mice responded when immunized with R32FT without proteosomes but the antibody response was greatly increased when R32FT was complexed to proteosomes. The response in rabbits receiving R32FT hydrophobically complexed to proteosomes was consistent with the murine results. The antibody response was also dependent on the ratio of R32FT to proteosomes and on the dose of recombinant protein given. These data show that a hypo-immunogenic, genetically-restricted protein can become immunogenic even in a previous non-responder mouse strain by genetically engineering it to contain a hydrophobic decapeptide carboxy-terminal anchor. Furthermore, these data demonstrate that proteosomes can further enhance the immunogenicity of a recombinant protein when complexed to it via its hydrophobic anchor. Since R32Ft is readily purified, well-expressed, and highly immunogenic when complexed to proteosomes, it is a prime candidate as a human vaccine component to enhance immunity against *P. falciparum* sporozoites.

- 80 FINE SPECIFICITY OF POLYCLONAL SERA RAISED AGAINST THE NON-REPEATING REGIONS OF THE *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN. M. C. Seguin*, M. R. Hollingdate, C. Silverman, G. F. Wasserman, M. Gross, and D. M. Gordon. Department of Immunology, Walter Reed Army Institute of Research, Washington DC, Biomedical Research Institute, Rockville, MD, Smith-Kline & French Laboratories, Swedeland, PA.

The repeating tetrapeptide region is the immunodominant B cell epitope of the circumsporozoite (CS) protein found on *Plasmodium falciparum* sporozoites. Although it has been shown that antibodies to analogous regions in rodent malaria models can confer protection in passive transfer experiments, very little information is available regarding the ability of antibodies to other regions on the CS protein to induce protection. We have produced a recombinant protein in *E. coli* which consists of amino acids 1 thru 105 and 296 thru 412 from the mature *P. falciparum* CS protein expressed in conjunction with 81 amino acids of the nonstructural protein (NS-1) from influenza A. This protein, referred to as NS181RLF59, was used to immunize rabbits and mice, producing high titer sera based on IFA and ELISA. Rabbit sera demonstrated varying degrees of inhibition of sporozoite invasion (ISI) activity, while mouse sera showed no ISI activity. Sera from these animals were then analyzed for fine specificity using polypropylene pins containing sequential hexapeptides from NS181RLF59, which overlapped by 5 amino acids. This analysis identified areas which may correlate with ISI activity.

ASTMH POSTER SESSION I

- 81 IN VITRO INHIBITION OF EXO-ERYTHROCYTIC SCHIZONT DEVELOPMENT BY SERA FROM SAIMIRI MONKEYS IMMUNIZED WITH PLASMODIUM VIVAX RECOMBINANT CIRCUMSPOROZOITE PROTEINS. *P. Millet, R. Rosenberg, W.E. Collins, R. Wirtz, and J.R. Broderson. Malaria Branch and Office of Scientific Services, Centers for Disease Control, Atlanta, GA; AFRIMS, APO, San Francisco, CA; and Walter Reed Army Institute of Research, Washington, DC.

Serum samples from Saimiri sciureus boliviensis monkeys immunized with 1 of 2 circumsporozoite (CS) recombinant proteins of Plasmodium vivax (VIVAX-1 or NS181V20) or with irradiated sporozoites of P. vivax, Salvador I strain, were tested for the ability to inhibit P. vivax, Salvador I strain, sporozoite development in primary cultures of Saimiri monkey hepatocytes. VIVAX-1 contained 60% of the entire length of the CS protein, including the repeat region; NS181V20 contained the repeat region only. Post-immunization sera inhibited sporozoite development by 85% to 100% compared to the pre-immunization sera, thus demonstrating the induction of antibodies effective against sporozoites; however, 100% inhibition rarely occurred. The samples were also tested with sporozoites from another strain of P. vivax that did not react with a monoclonal antibody against the repeat sequence of the Salvador I strain. Serum samples from monkeys immunized with the CS recombinant proteins in muramyl tripeptide did not inhibit schizont development; samples from monkeys immunized with irradiated sporozoites inhibited schizont development by 85% to 100%. These results indicate that sporozoite antigenic sites other than those contained in the recombinant CS proteins tested may induce protective antibodies. Supported in part by USAID PASA No STB-0453-23-P-HZ-00165-03 and done while PM held a National Research Council - Centers for Disease Control Research Associateship.

- 82 SAFETY AND IMMUNOGENICITY OF RECOMBINANT PLASMODIUM VIVAX SPOOROZOITE VACCINE IN VOLUNTEERS. *D. Herrington (1), E.J. Nardin (2), R. Bank (1) G.A. Losonsky (1), I. Bathurst (3), P.J. Barr (3), R.S. Nussenzweig (2), V. Nussenzweig (2), M.M. Levine (1). 1) Center for Vaccine Development, University of Maryland, Baltimore, MD; 2) Department of Molecular Parasitology and Department of Pathology, New York University Medical Center, New York, NY; 3) Chiron Corporation, Emeryville, CA.

A recombinant P. vivax circumsporozoite (CS) antigen containing 19 tandem amino acid sequences DRA(D/A)GQPAG, 15 amino acids preceding these repeats, and 48 amino acids flanking the repeats at their C-terminal end was expressed in yeast and adsorbed onto aluminum hydroxide for use as a malaria vaccine. In a Phase I study of safety and immunogenicity, 30 volunteers were divided into 4 groups of 5, 5, 10, and 10 and immunized by intramuscular injection with 50, 100, 200, or 400 mcg of vaccine, respectively.

Primary vaccinations and a 6 week booster immunization were well tolerated. Trivial tenderness at the vaccination site was noted by approximately 50% of volunteers. There were no systemic adverse effects.

Two weeks following the booster immunization, antibody and cell mediated immune responses were measured. All volunteers in the 400 mcg group, and 6 of 10 in the 200 mcg group generated IgG against P. vivax CS protein as determined by Western blot. However, the magnitude of the antibody response by ELISA titers was generally low. Antigen-driven lymphocyte replication studies failed to detect specific proliferative responses to the purified recombinant CS protein of P. vivax. These preliminary results suggest that while this P. vivax sporozoite vaccine is safe, the immunogenicity may not be optimal. A second booster immunization is planned for all dosage groups and additional tolerability and immunogenicity data will be presented.

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- 83 A CIRCUMSPOROZOITE-LIKE PROTEIN IS PRESENT IN MICRONEMES OF MATURE BLOOD STAGES OF MALARIA PARASITES. A. H. Cochrane*, S. Uni, M. Maracic, L. di Giovanni, M. Aikawa, and R. S. Nussenzweig. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY and Institute of Pathology, Case Western Reserve University, Cleveland, OH.

We demonstrate for the first time the presence of a circumsporozoite (CS)-like protein in invasive blood stages of malaria parasites. Immunogold electron microscopy using antisporeozoite monoclonal antibodies localized these antigens in the micronemes of merozoites. Western immunoblot and two-dimensional gel electrophoresis of mature blood stage extracts of *Plasmodium falciparum*, *P. berghei*, *P. cynomolgi*, and *P. brasilianum* identified polypeptides having the same apparent molecular mass and isoelectric points as the corresponding sporozoite (CS) proteins. The CS-like protein of merozoites is present in relatively minor amounts, compared to the CS protein of sporozoites. Mice with long-term *P. berghei* blood-induced infections develop antibodies which react with sporozoites. These and other findings will be discussed.

This work was supported by the Agency for International Development (DPE-0453-A-00-5012-00).

- 84 A SIMPLE DOT-IMMUNOBINDING TECHNIQUE FOR THE DETECTION OF *PLASMODIUM FALCIPARUM* SPOROZOITES IN MOSQUITOS. Gary W. Long, and John J. Oprandy, Naval Medical Research Institute, Infectious Diseases Department, Bethesda, MD.

We describe a simple technique to detect sporozoites of *Plasmodium falciparum* in mosquitos. This method relies on the adsorption of sporozoite antigens to a hydrophobic membrane PVDF. To perform the technique, mosquitos or pools of mosquitos are lightly ground in SDS buffer and forced by pressure through a nonadsorbing hydrophilic membrane sandwiched to the hydrophobic "capture" membrane. The first membrane removes debris while the second nonselectively captures the parasite antigens. The membrane can be processed immediately or stored and processed later. Detection is accomplished using a monoclonal antibody to the circumsporozoite protein and enzyme-labelled second antibody. The system consistently detects as few as ten sporozoites per mosquito. The advantages of the systems are: simplicity, it does not require custom labelled monoclonal antibodies, and membranes are less expensive and less bulky than microtiter plates. This technique could readily be applied to field use.

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- 85 DESIGNING PROTEOSOME-PEPTIDE VACCINES TO INDUCE ANTIBODIES AGAINST THE HIGHLY CONSERVED PENTAPEPTIDE N1a (KLKQP) OF MALARIA CS PROTEINS: EFFECTS OF REPLICATING THE HYDROPHOBIC ANCHOR AND/OR N1a. G.H. Lowell, L.F. Smith, M. Carter, S. Aley, R. Jaffe, R. Wirtz, P. Leland, V. Harrod, C. Schultz, W.D. Zollinger, D. Gordon, J.C. Sadoff, J. Chulay and M.R. Hollingdale. Walter Reed Army Institute of Research, Washington DC and The Biomedical research Institute, Rockville, MD.

The N1 region of CS proteins contains the putative sporozoite receptor for hepatocyte invasion since it binds saturably to hepatic cells, and anti-N1 antibodies blocked sporozoite invasion. The active binding site is contained within the highly conserved sequence N1a (KLKQP). We have shown that adding a hydrophobic anchor and cysteine to peptides and complexing them to meningococcal outer membrane proteosomes greatly enhances their immunogenicity. Vaccines were designed to contain 1-4 tandemly repeated N1a peptide and 1-3 tandem repeats of the hydrophobic heptapeptide anchor FLLALLF added to either the amino or carboxy terminal of (N1a). After two immunizations of outbred and Balb/c mice with 12 of the constructs the most immunogenic vaccines contained (N1a)4-(Ft)2 or (N1a)4-(Ft)1, and elicited antibodies that recognized *P. falciparum* and *P. berghei* sporozoites by IFA and ISI. Other constructs lacked significant activity. These data show that the proteosome-hydrophobic anchor system can be used to make a pentapeptide immunogenic and that the epitope:anchor ratio and orientation is critically important.

- 86 LOCALIZATION OF CS AND NON-CS ANTIGENS WITHIN *PLASMODIUM FALCIPARUM* EE PARASITES BY IMMUNOELECTRON MICROSCOPY. J.F.G.M. Meis, T. Ponnudurai, B. Mons, A. van Belkum, P. van Eerd, H. Schellekens, S.B. Aley and M.R. Hollingdale. Universities of Nijmegen and Leiden Medical Schools, Primate Center TNO, Rijswijk, The Netherlands; The Biomedical Research Institute, Rockville MD.

Mature *P. falciparum* EE parasites in chimpanzee liver were examined by immunoelectron microscopy six days after sporozoite inoculation. Using anti-CS repeat antibodies, we have previously shown that CS proteins are localized within *P. berghei* EE parasites on the parasite membrane and membranes of vesicles containing fluffy material. However, we have suggested that CS proteins may be processed during invasion. Thus CS non-repeat regions may be localized on other EE organelles. Rabbits were immunized with *P. falciparum* CS repeat, N- or C-terminal peptides, or with intact *P. falciparum* sporozoites. Rabbit anti-repeat antibodies reacted weakly with the EE parasite membrane, and strongly with Golgi bodies in *P. falciparum* EE merozoites. Rabbit anti-*P. falciparum* sporozoite antibodies reacted similarly. Of antisera to other CS regions, one, raised to a peptide representing a C-terminal sequence differed from anti-repeat antibodies and reacted with the fluffy material in the PVM. Rabbit antibodies to a peptide representing one repeat of the *P. falciparum* liver stage antigen (LSA) also reacted with the fluffy material, suggesting that this material contains LSA and possibly processed CS fragments and may be a target of cell mediated immunity.

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- 87 DIAGNOSIS OF T-CELL DETERMINANTS IN PLASMODIUM FALCIPARUM BY OLIGONUCLEOTIDE: DNA HYBRIDIZATION. A.A. Lal, V.E. do Rosario, K. Sakhuja, V. de la Cruz, M.R. Hollingdale and T.F. McCutchan. Biomedical Research Institute, Rockville, MD; NIH, Bethesda, MD.

Sequence analysis of the circumsporozoite (CS) protein of different strains of P. falciparum has revealed mutations leading to amino acid changes outside the repeat regions. These polymorphic regions in this vaccine candidate antigen cover the T-cell determinants which are important in vaccine design. We have used oligonucleotide probes specific for these mutations and PCR amplified CS gene fragments from 25 P. falciparum isolates from different geographical regions. We have found that nucleic acid hybridization is capable of discriminating single base substitutions to predict epitope structure. These results demonstrate that although this variation is localized in T-cell determinants, certain epitopes are common in P. falciparum isolates from distinct geographical regions. This may indicate that a limited number of T-cell epitopes may have a universal distribution.

- 88 PLASMODIUM GALLINACEUM: INHIBITION OF SPOROZOITE INVASION ISI AND EE DEVELOPMENT. E.M.M. da Rocha, A. Krettli, R. Gwadz, A. Appiah, M. Murphy, M.R. Hollingdale. Biomedical Research Institute, Rockville, MD; Fiocruz, Belo Horizonte, Brazil, NIAID, NIH, Bethesda, MD.

Mab's to CS repeat regions inhibit sporozoite invasion (ISI) of cultured cells. CS protein is also found in EE parasites and on EE merozoites, although mice immunized with sporozoites are susceptible to infection with EE merozoites. Whereas mammalian malarias undergo only one EE cycle, avian malarias develop EE merozoites that reinvade cultured cells. Recently, CS proteins of P. gallinaceum sporozoites have been identified, and Mab's to CS proteins inhibited P. gallinaceum sporozoite invasion of cultured chick macrophages. In vitro cultures of P. gallinaceum EE parasites have been established in a chick embryo cell line to study the significance of CS proteins in avian sporozoites and EE merozoites. P. gallinaceum sporozoites invaded and developed into EE trophozoites at 24 hr, EE schizonts containing merozoites at 38 hr, and a second type of larger EE parasite by 72 hr. CS proteins were strongly detected on sporozoites, EE parasites and merozoites, although only weakly on the 72 hr parasites. Mab's to CS strongly inhibited sporozoite invasion. The Mab's also reacted with P. berghei sporozoites by IFA, by western blot recognized the lower molecular weight processed form of P. berghei CS, and reacted in ISI with P. berghei sporozoites. ELISA assays using P. berghei CS peptides identified sequences of cross-reactivity suggesting a relationship between avian and rodent CS proteins

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- 89 IDENTIFICATION AND PURIFICATION OF A PLASMODIUM FALCIPARUM ANTIGEN PRESENT IN SPOROZOITES, EE PARASITES AND MEROZOITES. G. Chen, M.R. Hollingdale, S.B. Aley, P. Leland, D. Taylor. Biomedical Research Institute, Rockville, MD; Georgetown University, Washington, DC.

A monoclonal antibody (MAB E12) has been developed from a mouse infected with P. yoelii blood stages. The E12 MAB reacts by IFA with the apical region of Plasmodium falciparum and P. berghei sporozoites and merozoites, P. lophurae and P. gallinaceum blood stages, and P. berghei exoerythrocytic (EE) parasites, and had activity in the inhibition of sporozoite invasion of hepatoma cells. MAB E12 has been purified using a protein A column, and reacted by western blot using P. falciparum late stage schizonts. Using immunogold-silver staining or ¹²⁵I-labelled second antibody, one major band and one minor band were identified of molecular weights of 57 KD and 48 KD respectively. An affinity column has been constructed using purified MAB E12 coupled to Affi-Gel 10, and has been used to partially purify the 57 and 48 KD proteins from P. falciparum schizonts and merozoites. The immunogenic properties of E12 proteins in mice and rabbits will be discussed. The evolutionary conservation of this protein from avian to human malaria suggests it may play an important functional role in parasite development or function.

- 90 PLASMODIUM BERGHEI EXOERYTHROCYTIC ANTIGENS: SEROLOGY, CLONING AND GENE ANALYSIS. K. Sakhuja, B. Sina, J. Zhu, M.R. Hollingdale. Biomedical Research Institute, Rockville, MD.

We have shown that polyclonal antisera or MAB's to red blood cell (RBC) parasites react with exoerythrocytic (EE) stage parasites of P. berghei and P. vivax by IFA. Anti-RBC stage antisera immuno-precipitated and reacted in western blots with many antigens common to EE and RBC stages. MAB C2G1 was developed from mice immunized with P. berghei RBC stages and reacted with EE and RBC schizonts only during segmentation and merozoite formation. C2G1 immunoprecipitated a 250KD antigen from ³⁵S-methionine labelled P. berghei RBC parasites, and a similar 250KD antigen from ³⁵S-cysteine labelled P. berghei EE parasites and was processed to smaller weight fragments during merozoite release. A P. berghei genomic expression library was screened with MAB C2G1. One positive clone was obtained with C2G1 with an insert size of 6.8Kb, and is being sequenced. Using mouse polyclonal anti-RBC stage sera, about 70 clones were identified. The strongest reactive clone, SK47, was chosen for sequence analysis. SK47 clone has an insert size of 6 Kb and expresses a protein of about 80 KD under β -galactosidase promotion as shown by coomassie blue staining and western blot. SK47 protein has been purified from E. coli and semi-pure extracts used to immunize mice. Affinity purified antibodies are being used to localize the SK47 within P. berghei EE and RBC stage parasites. The P. berghei-mouse sporozoite challenge model will evaluate the protective activity of the C2G1 and SK47 antigens.

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91 HUMORAL IMMUNE RESPONSE TO THE CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM VIVAX IN RESIDENTS OF SEVERAL VILLAGES IN THE PERUVIAN JUNGLE.

E. D. Franke*, C. Lucas, H. Coveñas and E. San Roman.
U. S. Naval Medical Research Institute Detachment - Lima, Peru and Occidental Petroleum Corporation of Peru.

The prevalence of antibodies against the central repeat region of the circumsporozoite protein (CSP) of Plasmodium vivax was determined in residents of a malarious area in the jungle of northern Peru where the only malaria species infecting man is P. vivax. An ELISA was used to measure serum IgG antibodies against the recombinant CSP proteins NS1V20 (Smith Kline & French) and Vivax-1 (Chiron). The prevalence of IgG antibodies was highest in those individuals 15 years of age and older; 22% (31/141) had antibodies to NS1V20. Children below the age of 5 did not have antibodies to NS1V20. These data show that it may take several years of natural exposure to vivax sporozoites before antibodies against the CSP are produced. There was a positive correlation between the magnitudes of the responses to NS1V20 and Vivax-1 in individuals who had antibodies to NS1V20. However, only 53% (21/36) of those individuals who had IgG antibodies to NS1V20 also had IgG antibodies to Vivax-1. In addition, 2 of 16 negative control sera tested in the ELISA reacted strongly with NS1V20, but did not react with Vivax-1. These data indicate that some individuals may have antibodies to the portion of NS1V20 which contains the 81 amino acids from the non-structural protein 1 of influenza A and not the vivax CSP portion.

92 INCREASED IMMUNOGENICITY OF A RECOMBINANT PLASMODIUM FALCIPARUM SPOOROZOITE VACCINE, R32NS181, WHEN INCORPORATED INTO A LIPOSOME.

*J.E. Egan, C.R. Alving, R.L. Richards, G.F. Wasserman, C.C. Silverman.
Departments of Immunology and Membrane Biochemistry, WRAIR, Washington, D.C. and SK&F Laboratories, Swedeland, PA.

Protection from experimental Plasmodium falciparum (Pf) sporozoite challenge has previously been achieved by immunization with the recombinant vaccine, R32tet32. Increased immunogenicity was achieved by engineering a recombinant protein, R32NS181, in which the tet32 portion of the molecule was replaced with an 81 amino acid fragment of a nonstructural protein of Influenza A. Nonetheless, this increased immunogenicity was inadequate to provide protection from experimental Pf sporozoite challenge. To further enhance the immunogenicity of R32NS181, it has been incorporated into a liposome preparation which serves as a drug delivery system/adjuvant. Liposomes are spherical, lipid bilayer membrane vesicles. The specific preparation in this study is composed of a lipid membrane (consisting of dimyristoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, cholesterol and lipid A) and an aqueous compartment which contains R32NS181. The antibody responses in mice and rabbits were greatly enhanced by incorporation of R32NS181 into liposomes. Dose-response data for utilizing a liposome malaria vaccine candidate will be presented.

ASTMH POSTER SESSION I

- 93 BIOCHEMICALLY SIMILAR *LEISHMANIA* LIKE PARASITES FROM COLOMBIA AND PANAMA. RD Kreutzer*, M Groggl, DG Young, ED Rowton, RB Tesh, G Grimaldi Jr., & A Corredor. Youngstown State University, Youngstown, OH, Walter Reed Army Institute of Research, Washington, D.C., Univ. of Florida, Gainesville, FL, Yale Univ. School of Medicine, New Haven, CN, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil & Instituto Nacional de Salud, Bogota, Colombia.

Recently, Silveira et al. described a new peripylarian species which causes human dermal leishmaniasis in Brazil, *Leishmania* (Viannia) *lainsoni* (LL).

Enzyme profiles of parasites from 2 Colombian patients with typical dermal leishmaniasis (1980, 1981) indicated that the parasites were identical and distinct from all other *Leishmania* species. In 1985 and 1986 seven additional isolates, 2 from Colombia(CO) - *Lutzomyia hartmani* and 5 from Panama(PA) - 3 from *Lu. gomezi* and 1 each from *Lu. panamensis* and *Chloeopus hoffmanni*, were found to be peripylarian and biochemically very similar to the 2 isolates from humans and similar to LL. Diagrams (20 enzymes) which compare these and all peripylarian species will be presented. A crossbinding assay indicated CO and PA parasites were different from all other New World *Leishmania* spp. CO isolates (B4 & B13 monoclonals) could be assigned as a new parasite in the *L. braziliensis* complex. Both groups had similar and unique cross-reactivities with monoclonals against the *L. tropica* complex. The PA parasites showed differences in relation to the CO isolates, and there was no reactivity with any *L. braziliensis* monoclonals plus they had a distinct schizodeme fingerprint. These parasites grow well in a number of media including NNN and complete Schneider's. Genetic identities (I) were calculated from the enzyme data: CO-PA I = 0.914, CO-LL I = 0.667, PA-LL I = 0.547, CO or PA with any other *L. braziliensis* species I < 0.35. It appears that each might be a new *Leishmania* species. Supported by grants: WRAIR #DAMD-83-C-3119 & NIH #AI20208.

- 94 *LEISHMANIA INFANTUM SENSU LATO* ISOLATED FROM A GIANT RAT (*CRICETOMYS GAMBIANUS*) CAPTURED AT A CASE SITE OF HUMAN CUTANEOUS LEISHMANIASIS IN KENYA. P. Lawyer*, Y. Mebrahtu, P. Ngumbi, C. Anjili, and J. Nzovu. Kenya Medical Research Institute and U.S. Army Medical Research Unit, Nairobi Kenya

Investigations were conducted at a multiple-case site within a recently discovered rural focus of human cutaneous leishmaniasis. Whereas *Leishmania* isolates aspirated from patients at neighboring case sites adapted well to *in vitro* culture and were typed by cellulose acetate electrophoresis as *L. tropica*, the human isolate from this case site has been extremely difficult to culture in numbers sufficient for biochemical characterization. However, an isolate from one of 4 giant rats (*Cricetomys gambianus*) captured near the patients' house responded well to culture in Schneider's *Drosophila* medium and promastigotes were harvested for typing. Isozyme profiles of this isolate and reference strains of *L. donovani*, *L. infantum*, *L. tropica*, *L. major*, and *L. aethiopica* were compared by cellulose acetate electrophoresis using 11 enzymes (MDH, ME, ICD, 6PGD, G6PD, NH₁, NH₂, MPI, GPI, PGM₁, and PGM₂). Unexpectedly, the isolate was found to be indistinguishable from *L. infantum* for all enzymes tested. This is the first report of isolation of *L. infantum* in subsaharan Africa. An intense search for potential vector sand flies in the immediate vicinity of the house, and in a nearby cave where the family obtained their water, produced several sand fly species including *Phlebotomus duboscqi* and *P. guggisburgi*. The possible roles of these fly species in the transmission of cutaneous leishmaniasis caused by *L. infantum* and/or *L. tropica* are discussed.

ASTMH POSTER SESSION I

- 95 A RURAL FOCUS OF HUMAN CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA TROPICA* IN KENYA. Y. Mebrahtu*, P. Lawyer, G. Kirigi, J. Mbugua, G. Gachihi, K. Wasunna, H. Pamba, J. Sherwood, D. Koech, and C. Roberts. Kenya Medical Research Institute, U.S. Army Medical Research Unit. and University of Nairobi Medical Faculty, Nairobi, Kenya.

We have identified a focus of cutaneous leishmaniasis caused by *Leishmania tropica* in Kenya. Four indigenous Kenyans from Muruku Sublocation, Laikipia District, Rift Valley Province who have never traveled outside Kenya developed cutaneous lesions on the face and/or extremities which were found to contain *Leishmania* by smear and culture. Stationary-phase promastigotes from cultured isolates were analysed by cellulose acetate electrophoresis. Isozyme profiles of four isolates were compared with those of WHO reference strains of *L. donovani*, *L. infantum*, *L. aethiopica*, *L. major*, *L. tropica*, and *L. arabica* using 17 enzymes (LDH, MDH, ME, ICD, 6PGD, GAPDH₁, G6PD, HK, PFK, AK, ACP, NH₁, NH₂, MPI, GPI, PGM₁, and PGM₂) and were indistinguishable from the two *L. tropica* reference strains. All four case sites lie within a radius of four km. Several other suspected cases have been found in the same area and are being investigated.

- 96 ISOLATION OF *LEISHMANIA TROPICA* FROM *R. RATTUS* IN MALTA
*Bryce C. Walton, R. Killick-Kendrick, L. Gradoni, M. Gramiccia and D. Vella Briffa. Gettysburg, PA; Imperial College, Silwood Park, UK; Istituto Superiore di Sanita', Rome; Boffa Hospital, Floriana, Malta.

Visceral leishmaniasis (VL) has long been known to be endemic in the Maltese islands, but cutaneous leishmaniasis (CL) has only recently been recognized, with multiple cases diagnosed on the island of Gozo. One isolate had been made and the parasite was identified as *L. infantum* by isoenzyme analysis in London. CL is also known to be caused by *L. infantum* in other circum-Mediterranean localities. It is widely believed that *L. tropica* is maintained by human/sandfly/human transmission, and if it had existed on Malta, it was eliminated by widespread DDT spraying for malaria control, and it was postulated that *L. infantum* is the cause of CL now being transmitted in the Maltese islands. In a study of the epidemiology of *L. infantum* on the island of Gozo, *R. rattus* was considered a possible reservoir since isolates have been made in widely scattered areas over the globe. A total of 17 rats were live-trapped to search for parasites. Liver and spleen aspirates were taken aseptically for inoculation into NNN and USAMRU media and Giemsa stained smears of these organs examined directly. No amastigotes were seen in any smears, but 2/17 (11.7%) animals had promastigote growth in the cultures. These isolates were identified at the Istituto Superiore di Sanita' using six isoenzymes, PGM, PGI, GOT, G6PD, 6PGD, and HK. Profiles of both are identical to the WHO International Reference Strain for *L. tropica*. The possibility of CL due to this species in the Maltese islands, and of the role of *R. rattus* as a reservoir, require further investigation in view of this finding.

ASTMH POSTER SESSION I

97 THE DEVELOPMENT OF A SUBSPECIES-SPECIFIC RECOMBINANT DNA PROBE FOR THE CHARACTERIZATION OF LEISHMANIA MEXICANA MEXICANA (Lmm).

*J.M. Stiteler, P.V. Perkins, E.D. Rowton, and L.W. Roberts. Walter Reed Army Institute of Research, Washington, DC.

EcoRI restriction endonuclease fragments of kinetoplast DNA (kDNA) minicircles from a World Health Organization (WHO) standard Lmm isolate, MNYC/BZ/62/BEL21 (also designated WR#458), were ligated into the plasmid vector pUC8. The recombinant WR#458 kDNA-pUC8 plasmids were then used to transform the competent host E. coli strain, DH5-alpha. Transformant clones containing WR#458-pUC8 recombinant DNA plasmids were selected on the basis of ampicillin resistance and beta-galactosidase expression. Selected clones were then screened by DNA:DNA hybridization with whole kDNA probe from the parental isolate, WR#458 Lmm, to ensure that WR#458 DNA inserts existed within the recombinant plasmids of the clones. A second set of screenings of the selected clones with whole kDNA from the WHO standard MHOM/BZ/73/M2269 (Lm amazonensis [Lma], WR#669) were performed to identify clone(s) which specifically hybridize with WR#458 (Lmm) but did not cross-hybridize with WR#669 (Lma). One clone, pT10-11, was identified which contains WR#458-pUC8 recombinant DNA plasmids which do not cross-hybridize with WR#669 kDNA. Use of pT10-11 as a DNA probe against a battery of New World standard Leishmania isolates of both the L. mexicana and L. braziliensis complexes as well as a set of Leishmania isolates from Guatemala previously characterized as Lmm has demonstrated that pT10-11 has subspecies-specificity for Lmm isolates.

98 HUMAN T CELL CLONES SPECIFIC FOR DOMINANT LEISHMANIA ANTIGENS

*Steven G. Reed, Edgar M. Carvalho, Cynthia H. Sherbert, Kenneth H. Grabstein, and Warren D. Johnson, Jr., Seattle Biomedical Research Institute, Seattle, USA, University of Bahia, Salvador, Brazil, Immunex Corporation, Seattle, USA, and Cornell Medical College, New York, USA.

T cell lines and clones were generated from peripheral blood leukocytes (PBL) obtained from patients with clinical cases of visceral, cutaneous, or mucosal leishmaniasis, and from patients with chronic Chagas' disease (*Trypanosoma cruzi* infection). The cell lines and several of the clones from different patient types were tested for proliferation and cytokine production, using northern blots and bioassays, in response to leishmania antigens or to mitogens. Of twelve purified antigens of L. donovani cnagasi tested, two glycoproteins of Mr 30 and 42 were consistently most effective in stimulating proliferation and gamma interferon production in PBL from patients with cured visceral, cutaneous or mucosal leishmaniasis. Clones specific for each of these antigens, as well as clones which responded to both of these antigens were isolated from individuals recovered from visceral leishmaniasis. Approximately 30% of the T cell clones from visceral leishmaniasis patients responded to one or both of these antigens. Clones were found which produced IL-2, IL-4, and gamma interferon when stimulated with mitogen or parasite antigens. Of particular interest was the ability of these two antigens to elicit proliferation and gamma interferon production from PBL from patients with Chagas' disease. This study is important for the characterization of circulating T cells from individuals recovered from acute leishmaniasis, as well as in its approach to identifying antigens which may have important T cell epitopes.

ASTMH POSTER SESSION I

- 99 AN ENZYME IMMUNOSORBENT ASSAY (EIA) FOR THE SENSITIVE AND SPECIFIC DIAGNOSIS OF CHAGAS' DISEASE. THE USE OF AMASTIGOTE AND EPIMASTIGOTE ANTIGENS. *A.A. Pan, G.B. Rosenberg, M. Hurley, G. Schock, V. Chu, and A. Aiyappa. Abbott Laboratories, Abbott Park, IL.

Chagas' Disease is caused by the unicellular parasite *Trypanosoma cruzi* and is estimated to affect over 30 million in Latin America. The organism survives in the bloodstream and, therefore, the disease is often contracted by transfusion. For this reason, the serodiagnosis of this disease is essential in blood banks and for treatment. To develop a more sensitive and specific diagnostic test for Chagas' Disease, two *T. cruzi* antigens have been identified and purified. One antigen is a pure glycoprotein from amastigotes, and the other a crude soluble fraction from epimastigotes. When tested in an EIA format against xenodiagnosed positive sera, both reagents had sensitivities of 100%. The specificity of the antigens was determined using leishmania and malaria sera acquired from Africa and India, insuring that these samples did not contain antibodies to *T. cruzi*. The amastigote and epimastigote antigens were 100% specific, whereas immunofluorescence or hemagglutination kits from Latin America cross-reacted with several of these samples. In this preliminary investigation, the EIA with either the amastigote or epimastigote antigen appears to be at least as sensitive and significantly more specific than either immunofluorescence or commercially available hemagglutination assays in the serodiagnosis of Chagas' Disease.

- 100 ANTI-INTERFERON γ ANTIBODIES WITH DIVERSE SPECIFICITIES MODULATE NATURAL RESISTANCE OF C3H/HeN MICE DIFFERENTLY DURING THE FIRST WEEK OF INFECTION WITH *Leishmania major*.

D.A. Leiby*¹, D.S. Finbloom², R.D. Schreiber³, and C.A. Nacy¹. ¹Dept. of Cellular Immunology, Walter Reed Army Inst of Res, Wash, DC.; ²Food and Drug Administration, Division of Cytokine Biology, Bethesda, MD; ³Dept. of Pathology, Washington University, St. Louis, MO.

C3H/HeN mice are resistant to infection with *L. major*: within 2-3 weeks of infection, the parasite is cleared from cutaneous tissues. Treatment of mice with the anti-IFN γ antibody DB-1 at the time of parasite inoculation abrogates this natural resistance. When antibody treatment is delayed for 1 wk, however, the mice resolve their cutaneous infection. To determine more precisely the time that IFN γ is required for resolution of disease, we used two different monoclonal antibodies: (1) DB-1, a murine IgG1 antibody prepared against rat IFN γ which neutralizes both antiviral activity and macrophage activation, and (2) H-22.1, a hamster IgG1 antibody prepared against mouse IFN γ which is specific for the IFN γ receptor binding site, but which does not neutralize antiviral activity. C3H/HeN mice were treated with the 0.5 mg i.p. antibodies during the first week of infection; Control mice received a monoclonal antibody of the same IgG1 isotype or PBS. Mice treated with H-22.1 developed necrotic footpads within 3 weeks of parasite inoculation; amastigote numbers increased 80-fold over the initial inoculum in these infected tissues. Cutaneous lesions were observed regardless of the day on which H-22.1 treatment was initiated (days 1-5 post inoculation). Treatment of mice with DB-1 after the first day of infection, however, resulted in only a minimal increase in footpad depth which rapidly returned to normal levels. Thus, H-22.1 is more efficient than DB-1 at abrogating natural resistance in C3H/HeN mice.

ASTMH POSTER SESSION I

101 LOCALIZED CUTANEOUS LEISHMANIASIS (CHICLERO'S ULCER) IN MEXICO. ----- SENSITIVITY AND SPECIFICITY OF ELISA FOR IgG ANTIBODIES TO LEISHMANIA - MEXICANA MEXICANA.

Garcia, M.R.*; Andrade, F.; Esquivel, R.; Simmonds, E.; Canto, S. & Cruz, A.L.
University of Yucatan (Mexico) Tropical Diseases Research Unit. Reference ----
Center for Leishmaniasis Control.

A total of 223 sera from human beings were processed by ELISA for IgG to -----
Leishmania mexicana mexicana promastigotes. Sera were classified in four -----
groups. A: 75 from healthy persons with Montenegro skin test negative, B: 74 -
from patients with Chiclero's Ulcer; C: 56 from healthy persons with Montene-
gro skin test positive ("infected"); D: 18 from patients with Chaga's disease
(7), Toxoplasmosis (4), Malaria (3), Mucosis (2), and Carcinoma (2). An -----
absorbance value 0.315 at 490 was considered positive. The sensitivities ---
were of 86% in acute cases and 54% in chronic cases respectively. There was an
absence of reactivity in the "infected" group. Cross reaction was observed in
6/7 (85%) sera from patients with Chaga's disease. The specificity was of 95%.
We can conclude that this method is useful to support the diagnosis of -----
Chiclero's Ulcer.

102 SERODIAGNOSIS OF HUMAN AND CANINE LEISHMANIASIS BY A RADIOIMMUNOASSAY. M.V.Londner¹, A.G.El Missiri², G.Rosen¹, T.A.Morsy², S.M.Afram², S.El Said² and S.H.Giannini³. 1.The Kuvim Centre, Hebrew University-Hadassah Medical School, Jerusalem, Israel. 2.Ain Shams University Research and Training Center on Vectors of Diseases, Cairo, Egypt. 3.Center for Vaccine Development, University of Maryland, School of Medicine, Baltimore, USA.

The first part of this study was undertaken to determine whether chronic
infection with Schistosoma mansoni, hyperendemic in parts of Egypt, would gen-
erate antibodies which might cross-react in three widely used serodiagnostic
tests for leishmaniasis: the indirect hemagglutination test(IHT), the radioim-
munoassay(RIA) and the dot enzyme immunosorbent assay(dot ELISA). Sera were col-
lected from 28 cases of cutaneous leishmaniasis and 25 cases of schistosomiasis
mansoni in Egypt. The sensitivity and specificity for each test was: IHT: 64% and
72%, dot ELISA: 75% and 88%, RIA 89% and 100%, respectively. This study shows that
detection of anti leishmanial antibodies by the RIA or the dot ELISA may be
used to differentiate patients with cutaneous leishmaniasis from those chron-
ically infected with S.mansoni in areas where this disease is endemic.
In the second part of this study the RIA was used in a serological study of
dogs from a visceral leishmaniasis (L.donovani infantum) endemic area. Sera were
collected from 58 dogs with leishmaniasis, 60 control dogs, 15 dogs with leptos-
pirosis, 28 dogs with toxoplasmosis and 7 dogs with filariasis. The sensitivity
of the RIA was 94% and the specificity 100%. This study shows that the RIA has
a very high diagnostic accuracy to detect canine leishmaniasis in an endemic
area.

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Epidemiology and Control of Arthropod-Borne Diseases in the Middle East.

ASTMH POSTER SESSION I

103 A RAPID AND RELIABLE SERODIAGNOSTIC TEST FOR ACUTE VISCERAL LEISHMANIASIS

*Jeannine M. Scott, Wayne G. Shreffler, Roberto Badaro, and Steven G. Reed, Seattle Biomedical Research Institute, Seattle, USA, and Federal University of Bahia, Salvador, Brazil

A diagnosis of acute visceral leishmaniasis, kala-azar, may be complicated by symptoms resembling other infectious diseases such as malaria, typhoid, or schistosomiasis. These diseases often overlap in distribution. Attempted identification of leishmania amastigotes in spleen or bone marrow is commonly relied upon for definitive diagnosis of visceral leishmaniasis. However, if laboratory tests are unavailable or unreliable, this procedure may not be useful. Biopsy of lymphoid tissue is often painful and dangerous to the patient, and considerable skill is required to perform the procedure and read the slide. Other methods of parasite identification are relatively slow, expensive, and/or not readily adapted for field use.

Properly performed, serological tests can readily differentiate acute visceral leishmaniasis from other infectious diseases. Most commonly used are the micro ELISA or the dot ELISA. We have developed a serodiagnostic test which is easier and more practical than ELISA methods. In this test, antigen is placed on plastic strips and dried. The antigen sensitized plastic is stable and requires no refrigeration. The strip is incubated with diluted serum for 30 min., washed, and incubated with protein A-gold conjugate for 30 min., at which time a visible red precipitate will form where antibody binding has occurred. When the test was used with serum obtained from cases of both Old and New World kala-azar, it was shown to be 100% sensitive. The test distinguishes kala-azar sera from those obtained from individuals with mycobacterial infections, cutaneous leishmaniasis, cured visceral leishmaniasis, sub-clinical leishmaniasis, or other tropical infections. Thus it provides a rapid and reliable way to distinguish kala-azar from other infections, and is suitable for field use with minimal training. The materials used are stable without refrigeration, and are relatively inexpensive on a per test basis. The test can be performed in volumes of serum obtained from a finger stick.

104 COMPARISON OF KETOCONAZOLE TO PENTOSTAM AND TO PLACEBO IN THE TREATMENT OF LEISHMANIA BRAZILIENSIS PANAMANSIS CUTANEOUS LEISHMANIASIS: A FINAL REPORT . R.E. Saenz, H. Paz, J.D. Berman*. Gorgas Mem Lab, Panama; WRAIR, Washington, D.C.

Purpose and Methods: The classical agent for cutaneous leishmaniasis is pentavalent antimony. However, there are no reports of the efficacy of antimony vs placebo, or of the efficacy of any alternative therapy vs either antimony or placebo. In the present study, the oral antifungal agent ketoconazole (600 mg/day for 28 days) was compared to a recommended regimen of intramuscular Pentostam (20 mg antimony/kg, with a maximum of 850 mg antimony/day, for 20 days) in a randomized study of the treatment of Panamanian cutaneous leishmaniasis due to *Leishmania braziliensis panamensis*. The 850 mg maximum daily dose of antimony meant that Pentostam patients received a mean of 13 mg antimony/kg/day. A separate group of patients with this disease was administered placebo. Patients were followed for 12 months after the end of treatment.

Results: Ketoconazole clinically cured 16/21 = 76% of patients. The lesions on 9 patients healed by 1 month after therapy and on the other 7 patients healed by 3 months after therapy. Side effects were limited to a 27% incidence of mild, reversible hepatocellular enzyme elevation and an asymptomatic, reversible ~70% decrease in serum testosterone in all patients. Pentostam cured 13/19 = 68% of patients; the lesions on 7 patients healed by the end of therapy and on 4 other patients by 1 month after the end of therapy. Side effects were a 47% incidence of mild, reversible hepatocellular enzyme elevation and the morbidity due to 20 intramuscular injections in almost all patients. The placebo group of 11 patients had a 0% cure rate. By 1 month after therapy, all placebo patients demonstrated new lesions or one lesion that was 23-875% larger than pre-therapy.

Conclusions: Both ketoconazole and Pentostam were more effective than placebo against *L. braziliensis panamensis* cutaneous leishmaniasis. Oral ketoconazole is at least as effective as this Pentostam regimen and can be recommended as an initial treatment for this disease.

ASTMH POSTER SESSION I

- 105 CLINICAL RELEVANCE OF IN VITRO ANTILEISHMANIAL SUSCEPTIBILITY TESTING. M. Grogl*, T.N. Thomason, D. Panisko, J.S. Keystone, W.A. Reid and D.E. Kyle. Walter Reed Army Institute of Research, Washington, D.C. and Toronto General Hospital, Toronto, Ontario, Canada.

Recently we have developed and adopted a semiautomated microdilution technique (SAMT) as the standard *in vitro* technique for determining the susceptibility of *Leishmania* isolates to antileishmanial drugs and for evaluating the activity of putative antileishmanial agents (Grogl *et al.*, 1989). For example, patterns of susceptibility of isolates from patients with different clinical outcomes are listed in the following table.

SPECIES	ISOLATE	DRUG	CLINICAL OUTCOME	SAMT-IC ₅₀ (+ 1SD)	
				Pentostam (mg/ml)	Ketoconazole (ug/ml)
<i>Lm</i>	WR 840	Glucantime	Unresponsive	0.89 (+.007)	0.84 (+.006)
<i>Lp</i>	WR 739	Pentostam	Responsive	0.04 (+.002)	
<i>Lb</i>	WR 708	Pentostam	Responsive	0.04 (+.001)	
<i>Lg</i>	WR 930	Itraconazole	Unresponsive	0.55 (+.003)	15.74 (+1.3)
<i>Lg</i>	WR 931	Itraconazole	Unresponsive	0.29 (+.001)	1.76 (+.21)
<i>La</i>	WR669-C15	Pentostam	Control	0.06 (+.007)	
<i>Lp</i>	WR746-C19	Pentostam	Control	0.06 (+.01)	0.66 (+.009)

Interestingly, we have correlated subcurative treatment to a decrease in drug sensitivity in a patient (WR840) with cutaneous leishmaniasis who received a total of 96 ampules of glucantime during a period of 4 years (1 ampule = 425 mg Sb). In contrast, a *Lb* strain (WR 843) isolated before treatment was found to be moderately resistant to Pentostam. These results indicate that in nature, in the absence of drug pressure, there are populations of parasites that substantially differ in their susceptibility to Pentostam.

- 106 COMPARATIVE ANALYSIS OF DIFFERING SENSITIVITIES TO PENTOSTAM OF TWO SPECIES OF *LEISHMANIA* BY PULSE-FIELD GEL ELECTROPHORESIS. R.K. Martin*, M. Grogl, N.A. Edwards, and D.E. Kyle. Walter Reed Army Institute of Research, Washington, D.C.

We have developed clones of *L. amazonensis* (WR 669-C1 4) and *L. panamensis* (WR 746-C1 9) that exhibit increased resistance to pentavalent antimonials. A comparative analysis of these sensitive and resistant clones by electrophoretic karyotyping was undertaken to ascertain inter- and intra-species differences. Pulse-field gel electrophoresis (PFGE) separated 20 and 17 dominant chromosomes of *L. amazonensis* (C14) and of *L. panamensis* (C19), respectively. The most notable interspecies differences were observed in the 200 kb to 900 kb range. The molecular weight range of chromosomes for sensitive and resistant clones of WR 669 and WR 746 was from <200 kb to >2000 kb. Clones of WR 746 that were resistant to pentostam, methotrexate, and arsenic acid were found to have a more intensely stained chromosome (184 kb) than susceptible parasites. PFGE provides a convenient means of identifying the chromosomal changes of *in vitro* induced drug-resistance in parasites.

ASTMH POSTER SESSION I

107 QUANTITATIVE IN VITRO DRUG SUSCEPTIBILITY TESTS OF
LEISHMANIA SPP. FROM PATIENTS UNRESPONSIVE TO
PENTAVALENT ANTIMONY (SBV) THERAPY.

*J.E. Jackson, J.D. Tally, Y.B. Mebrahtu, P.G. Lawyer, J.B.O. Were, S.G. Reed, Div. Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; U.S. Army Medical Research Unit, Nairobi, Kenya; and Seattle Biomedical Research Institute, Seattle, WA.

Fourteen leishmanial isolates from human patients were evaluated for SbV drug susceptibility using a new in vitro procedure (Jackson and Tally, 1989, Am J Trop Med Hyg, In Press). Seven of the isolates were tested "blind", i.e. without known patient history. Of the 7 "blind" tests, 3 clinical histories were available (4 others because of on-going therapy are not yet available). The 3 "blind" test results were from 1 cutaneous and 2 visceral patients whose disease manifestations were unresponsive to several "courses" of SbV therapy. In corresponding in vitro results, parasite resistance to 600 ug/ml Sb (or 100X Sb levels achievable in human serum) was evident. For contrast, a South American visceral parasite clinically cured with one "course" of Glucantime was in vitro sensitive at 3 ug/ml Sb. In all known cases (10/14), in vitro results correlated well with patient treatment history and provided a quantitative assessment of parasite SbV-sensitivity at Sb levels below those considered maximal in human serum.

108 IN VITRO QUANTIFICATION OF LOT-TO-LOT VARIABILITY IN
ANTILEISHMANIAL ACTIVITY OF PENTAVALENT ANTIMONIALS.

*J.E. Jackson, J.D. Tally, and W.Y. Ellis, Departments of Parasitology and Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Pentavalent antimonials (SbV), drugs-of-choice for treatment of human leishmaniases, have been reported to lack "defined chemical formulae, and to vary somewhat in activity [in man], toxicity, and Sb-content batch-to-batch" (Marsden, 1985, Rev Soc Brasil Med Trop 18:187-198). Even the British Pharmacopoeia defines Pentostam based on drug lot activity and toxicity within a 30-34% range of Sb-content. Using a new in vitro drug test procedure (Jackson and Tally, 1989, Am J Trop Med Hyg, In Press), 5 different production lots of Pentostam and Glucantime were quantitatively compared. We detected up to a maximum of 30X difference in antileishmanial activity among drug lots. Differences in activity were independent of drug type (Pentostam or Glucantime), or Sb-content. These data may be explainable based on differential carbohydrate moiety polymerization inherent to the formulation of these 2 SbV drugs (E. Steck, personal communication). This in vitro procedure appears to offer clinicians a rapid method for evaluating the activity of antileishmanials before therapy begins.

ASTMH POSTER SESSION I

- 109 CHARACTERIZATION OF A SCHISTOSOMA MANSONI FEMALE-SPECIFIC GENE THAT PUTATIVELY ENCODES A 48 KDA EGGSHELL PROTEIN. *L. Chen, H. Hirai, D.M. Rekosh and P.T. LoVerde, Department of Microbiology, SUNY, Buffalo, NY

Egg production by worm pairs is a major cause of pathogenesis in schistosomiasis. Thus an understanding of female reproductive development, especially mechanisms of female-specific gene regulation is of major importance in disease control. Previous studies have suggested that at least two proteins are involved in S. mansoni eggshell formation: a 14 kDa glycine-rich protein (p14) and a 48 kDa tyrosine and histidine-rich protein (p48). The gene that encodes p14 has been fully characterized in our laboratory. A previously isolated 600 bp cDNA that encodes the carboxy end of p48 was used to identify and isolate a recombinant phage containing an entire copy of the p48 gene. The gene is contained on a 2.8 kbp PstI/XbaI fragment. Sequence analysis reveals 3 open reading frames, one of which encodes a deduced polypeptide of 44 kDa that shows a strong homology to chorion proteins of insects. There are no introns. The cap-site of the mRNA was determined by primer extension using RNA as template and dideoxy sequencing. The upstream regions of the gene contain putative cis-acting elements (TCACGT, GTAGAAT) that have been shown to play a role in the regulation of chorion gene expression in insects. Northern analysis of RNA isolated from different developmental stages shows that mRNA encoding p48 is detectable only in mature female worms and the ability to detect mRNA coincides with egg production. Genomic Southern analysis and *in situ* hybridization data indicate that the p48 gene is present in a few copies on chromosome 3. Our data is consistent with the notion that p48 is an eggshell protein. (Supported by AI27219).

- 110 Schistosoma mansoni LARVACIDAL ACTIVITY OF MURINE BRONCHOALVEOLAR LAVAGE CELLS.

F.A.Lewis*, C.A.White, J.E.Ball, and G.M.Niemann. Biomedical Research Institute, Rockville, MD.

Most evidence indicates that elimination of challenge Schistosoma mansoni larvae in irradiated cercarial-immunized mice occurs during, or shortly after, the lung migration phase. We examined the ability of broncho-alveolar lavage cells (BACs) from normal and immune mice to kill mechanically-derived schistosomules *in vitro*. High levels of larvacidal activity were found in cultures of BACs from C57Bl/6 (B6) mice. On a cell-to-target basis, equivalent larvacidal activity was found in cell cultures from normal and immune mice. BAC-mediated killing was 2-3 fold more efficient than killing mediated by cells obtained from the peritoneal cavity. The level of BAC-mediated killing was strikingly mouse strain-dependent. BACs from two mouse strains with known macrophage defects (A/J and P/N), possessed little larvacidal activity. Cells of the IC-21 macrophage cell line were much less active than B6 BACs. Incubation of IC-21 cells in lymphokine-containing supernates increased larvacidal activity, but had little enhancing effect on the killing activity of B6 BACs. Cytocentrifuge preparations revealed that macrophages comprised 85-95% of BACs from all mouse strains tested. These results show the extremely efficient larvacidal activity of BACs from B6 mice, and indicate the potentially important role these cells may play in normal- and immune-mediated attrition of schistosomes passing through the lungs during the course of their normal migration. (Supported by NIH AI16006).

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IMMUNIZATION OF MICE WITH THE MAJOR LYMPHOPROLIFERATIVE PEAK OF FRACTIONATED SOLUBLE EGG ANTIGEN (SEA) INDUCES A HUMORAL RESPONSE TO BOTH CARBOHYDRATE AND POLYPEPTIDE EPITOPES.

John J. Quinn and Donald A. Harn. Department of Tropical Public Health, Harvard School of Public Health, Boston, Ma. 02115.

Soluble egg Ags (SEA) were fractionated according to charge by agarose-isoelectric focusing (A-IEF) in order to determine those antigens which elicit the granulomatous response in *S. mansoni*. Ag in the acid pI (3.0-5.0) range consistently elicited a stimulatory lymphoproliferative response to acute and chronically infected mice, the major constituent peak being pI 4.6-4.8. Mice were immunized with fractions corresponding to pI 4.6-4.8 from gels run either with SEA or DDH2O.

Serum from the SEA immunized mice showed binding similar to that of a protective monoclonal (E.1) by Elisa and Western blot, recognizing the 200,38,17 kD family of antigens which have been shown to bind to carbohydrate epitopes. Adsorption of pI fraction 4.6-4.8 with E.1 coupled to Protein A-sepharose resulted in a 99% reduction of E.1 binding, 78% reduction of immunized mouse sera, and 48% reduction of binding of chronic mouse sera. Sodium metaperiodate oxidation also resulted in decreased binding of antiserum or monoclonals, suggesting that antibodies raised against pI fraction 4.6-4.8 recognize polypeptide epitopes in addition to the carbohydrate epitopes seen by the protective monoclonal antibody.

Subsequently, monoclonal antibodies were raised against the major lymphostimulatory peak (pI 4.6-4.8). Interestingly, the majority of secreting mAbs were of isotype IgM with lamda light chains. Western blot analysis of these mAbs shows similar binding to the 200, 38, 17 kD family seen by E.1 but an additional band was seen at 70-80 kD.

- 112 **TRANSMISSION OF LYMPHOCYTE RESPONSIVENESS TO SCHISTOSOMAL ANTIGENS BY BREAST FEEDING.** Ahmed M. Eissa, Mohamed A. Saad, Aida K. Abdel Ghaffar, Ibrahim M. El-Sharkawy and Karim A. Kamal*. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt and Department of Pediatrics, Medical School, Al-Azhar University, Cairo, Egypt.

Fifty healthy infants were evaluated for the influence of their feeding patterns on the mother-to-child transmission of lymphocyte responsiveness to *Schistosoma mansoni* antigens. Positive delayed-type hypersensitivity reactions (DTH) were observed in 56% (14 out of 25) of infants who were breast fed by infected mothers. Two out of 10 formula fed infants born to infected mothers had positive DTH. A positive DTH was detected in only one infant out of 15 breast-fed by schistosomiasis-free mothers. These results suggest that cellular hypersensitivity to *S. mansoni* antigens can, at least in part, be transmitted in the colostrum/milk of infected mothers to their infants. (Supported by NMRDC, Bethesda, MD, Work Unit # 3M161102BS13.AK. 311).

ASTMH POSTER SESSION I

- 113 SCHISTOSOMA MANSONI INFECTION REDUCES THE PERCENT OF VIABLE LITTERS FROM PREGNANT CBA MICE AND ALTERS IMMUNE RESPONSIVENESS OF THE OFFSPRING.

*T. Amano, G.L. Freeman, Jr., and D.G. Colley. Dept. Microbiol., Vanderbilt Univ. Sch. Med. and VA Med. Ctr., Nashville, TN.

This study was done to see if murine schistosomiasis mansoni alters pregnancy and/or influences immune responses in offspring of infected (INF) mothers. Effects could be due to exposure to idiotypes (Ids) on anti-parasite-related antibodies or circulating antigens, and might be manifest neonatally, or anamnesticly, on subsequent infection. We determined litter production, monitored transfer of anti-egg antigen (SEA) antibody to offspring and induction of anti-SEA immune responses in offspring, and examined responses after S. mansoni infection. Pregnancies (133) of INF CBA/J mice led to only 34% litters that survived ≥ 1 week. In contrast, 77% of 132 pregnancies of unINF CBA/J mice resulted in viable litters. Offspring of INF mothers received high titers of anti-SEA IgG antibody, detectable for 12 weeks. Very low titers of anti-SEA IgM were seen early in life. Spleen cells of some offspring of INF mothers responded to anti-SEA Ids. Spleen cells of most offspring of INF mothers responded to SEA earlier than did cells from offspring of unINF mothers. Also, egg-induced granulomas in mice born to INF mothers attained maximal size earlier, and modulated earlier, than those from unINF mothers. (Supported by: Veterans Administration, AI-11289)

- 114 Percutaneous compared to per-oral Schistosoma mansoni infections in hamsters

Peter G. Kremsner, Slavko Mravak, Gaby Poggensee
State Institute of Tropical Medicine, Berlin (West-Germany)

As there is a contradictory opinion about transmucosal penetration by cercariae, the efficacy of the percutaneous versus the per-oral route of Schistosoma mansoni infections was compared in hamsters. Three hamsters were infected percutaneously during an one hour both in water containing 1200 freshly hatched cercariae of S. mansoni. Three anaesthetized hamsters were infected perorally via pipetting the same number of cercariae into the mouths. To obtain quantitatively S. mansoni adults, liver perfusion of each hamster was performed 7 weeks after infection. From the percutaneously infected hamsters 350, 380 and 500 adult worms were hatched. Whereas from the per-orally infected animals 70, 110 and 300 adult worms were hatched. However, no differences of egg density could be obtained in liver sections. Although the parasite load was smaller after per-oral infection, this route of infection must be considered in naturally occurring schistosomiasis mansoni infections.

ASTMH POSTER SESSION I

- 115 DEVELOPMENTALLY REGULATED EXPRESSION OF SCHISTOSOMA MANSONI GLYCOPROTEINS. Bärbel Köster and Mette Strand. Johns Hopkins University School of Medicine, Department of Pharmacology and Molecular Sciences, Baltimore, MD 21205

In schistosome-infected hosts the humoral response is predominantly directed against carbohydrate epitopes. In order to examine the role of these immunogenic epitopes, we asked the question, whether they are unique to a given developmental stage or differentially expressed in the life cycle. By use of four monoclonal antibodies (mAb) recognizing distinct immunogenic epitopes previously described by us, the following pattern of expression was observed:

mAb	miracidia	cercariae	so.mules	adult worm	egg	
128C3	---	S	S	I		S = Surface I = Internal
2C1E4	---	S	S	I		
504B1			S	S		
503C3				S		

Mab 503C3 precipitated a single band of 60 kDa from adult worm extract; that antigen appears to be a membrane protein. The other mAbs recognized carbohydrate epitopes expressed on polypeptide backbones of different molecular sizes (□). These data were obtained by immunoprecipitation of glycoproteins metabolically labeled with [³⁵S] methionine or [¹²⁵I] surface-labeled proteins, by immunoelectronmicroscopy or immunofluorescence. The involvement of carbohydrate epitopes in the immunopathology and immunoprophylaxis is well established; the significance of the expression of stage specific glycoproteins needs further elucidation. This work was partially supported by the German Academic Exchange Service (DAAD, FRG)

- 116 THE IMMUNE-DEPENDENT ACTION OF PRAZIQUANTEL: MOLECULAR CHARACTERIZATION OF A SCHISTOSOMA MANSONI TARGET ANTIGEN. *Traci M. Tanaka and Mette Strand. Johns Hopkins University School of Medicine, Department of Pharmacology and Molecular Sciences, Baltimore, MD 21205

PraziquanTEL (PZQ) is the drug of choice for human schistosomiasis. The effectiveness of the drug has been shown to be impaired in schistosome-infected mice that have been depleted of B or T lymphocytes, suggesting that PZQ and the immune system work in synergy to eliminate schistosomes. This hypothesis is supported by the recent observation that transfer of a monoclonal antibody, mAb 305, at the time of PZQ treatment to mice that have been depleted of B lymphocytes restores the effectiveness of PZQ to its normal level. This mAb recognizes a 200 kDa glycosylphosphatidylinositol (GPI)-linked protein Schistosoma mansoni that becomes exposed on the surface of adult worms following treatment with PZQ.

The mechanism of interaction between the drug and the immune system is not known. One means of analyzing this interaction is the characterization of the target antigens recognized by the antibodies involved in the synergy between PZQ and the immune response. For this purpose, we used a rabbit polyclonal antiserum recognizing the 200 kDa antigen to isolate clones from an adult worm cDNA library expressed in lambda gt11. The cDNA insert hybridized to a 6.3 kb mRNA species, as determined by Northern analysis. Approximately one-third of the cDNA insert has been sequenced to date. The amino acid sequence deduced from this nucleotide sequence showed no similarity to any known protein sequences. Also, since the 200 kDa antigen is GPI-linked and PZQ modulates the surface accessibility of the protein, we are examining the interaction between the lipid anchor and PZQ.

ASTMH POSTER SESSION I

117 ANTI ELASTASE ANTIBODIES AS SERO-EPIDEMIOLOGICAL INDICATORS OF EXPOSURE TO TRANSMISSION OF SCHISTOSOMIASIS.

C.J. Shiff, Johns Hopkins University; Adeleh Esfandiari, Charles R. Drew Post-Graduate Medical School, Los Angeles; G. Kinoti, University of Nairobi and J. McKelrow, UCSF San Francisco.

Measurement of transmission patterns by seroepidemiology have been difficult with schistosomiasis because of the chronic nature of the infection and the broad nature of antigenic stimulus arising. However, elastase is produced only by cercariae and any immune response mounted against this antigen is indicative of cercarial challenge. The response is primarily IgM and of short duration.

We have examined the immune response of a group of Kenyan school children from the Mwea Irrigation Development area. Blood (fingerprick) specimens were obtained at the end of the summer transmission season when cercarial densities are high in natural waters. Urine and stool specimens were also taken to detect active infection. ELISA tests were run using plates sensitised with cercarial elastase, crude cercarial antigen and crude whole worm antigen (*S. mansoni*).

Results of a single cross-sectional study are reported. These will be of value in determining which persons were recently exposed to cercariae.

118 EOSINOPHILIC MENINGOENCEPHALITIS DUE TO ANGIOSTRONGYLUS CANTONENSIS AS THE CAUSE OF DEATH IN CAPTIVE NONHUMAN PRIMATES.

*C.H. Gardiner, S. Wells, A.E. Gutter, L. Fitzgerald, D.C. Anderson, R.K. Harris, and D.K. Nichols. Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, D.C.; Audubon Park and Zoological Gardens, New Orleans, LA; Ardastra Gardens and Zoo, Nassau, Bahamas; Yerkes Regional Primate Research Center, Emory University, Atlanta, GA; and Zoo/Path, P.O. Box 130, Bowie, MD.

Fatal eosinophilic meningoencephalitis due to Angiostrongylus cantonensis is reported in captive nonhuman primates. A howler monkey (Alouatta caraya) housed at the Audubon Park and Zoological Gardens, New Orleans, LA, died 21 days after initial clinical symptoms. A white-handed gibbon (Hylobates lar) died at the Ardastra Gardens and Zoo, Nassau, Bahamas, 17 days after onset of symptoms. Both nonhuman primates had access to free-ranging gastropods within the zoos. These are the first reported cases of natural infection by Angiostrongylus cantonensis in nonhuman primates in the western hemisphere and extends the distribution of this parasite to the Bahamas.

ASTMH POSTER SESSION I

- 119 ADULT MALE CAPILLARIID INFECTING THE ORAL MUCOSA OF MAN. *R.D. Neafie and A.J. Strano. Armed Forces Institute of Pathology, Washington, D.C. and St. John's Hospital, Springfield, Illinois.

A 63-year-old white female, who had been a lifelong resident of central Illinois, presented to the hospital with migratory pain and swelling in the oral cavity of several weeks duration. She was examined by an ENT surgeon who biopsied a suspect lesion.

Microscopic examination of the left alveolar ridge, revealed a nematode within the squamous mucosa which was provoking an inflammatory infiltrate. The worm had a rather uniform diameter of 50 μ m. The anterior portion of the worm contained a stichosome. The intestine, testis, vas deferens containing spermatozoa, ejaculatory duct, and spicular sheath were identified.

These morphologic features are those of the genus Capillaria and this worm was identified as an adult male Capillaria. Worms very rarely invade the oral mucosa of man and, to the best of our knowledge, this is the first reported case of Capillaria infecting the oral mucosa. The morphologic features of the parasite, its taxonomic position in relationship to other similar nematodes, and the histopathologic changes it has provoked will be illustrated.

- 120 MECHANISTIC STUDIES IN THE TRANSCUTICULAR DELIVERY OF ANTIPARASITIC DRUGS: BIOPHYSICAL TRANSPORT PROPERTIES OF THE CUTICLE OF THE PARASITIC NEMATODE ASCARIS SUUM.

*D.P. Thompson, N.F.H. Ho, T.G. Geary, T.J. Raub and C.L. Barsuhn. The S. John Company, Kalamazoo, MI.

To provide quantitative and mechanistic insight into drug transport properties of gastrointestinal nematodes, mass transport studies were conducted using isolate cuticle from Ascaris suum. Cuticle sections were mounted in two-chamber diffusion cells and steady-state rates of flux were determined for a number of radio-labelled permeants which varied in molecular size, lipophilicity and electrical charge. The permeability coefficient of the collagen matrix (lipid-extracted cuticle) vs molecular radius relationship showed the interdependence of molecular size and electrical charge of the permeants with respect to the aqueous pores of the negatively charged matrix. The permeability of neutral solutes decreased monotonically with size. Protonated amines permeated the aqueous pores faster than neutral solutes of comparable size while the permeation of anions was slower. The average pore size was estimated to be 15A⁰ in radius. The effective permeability coefficient of the nonlipid-extracted cuticle was delineated into the permeability coefficients of the water-filled collagen matrix and the lipoidal component of the cuticle to determine which layer was the rate-controlling barrier. While each solute was capable of penetrating the water-filled collagen matrix, the rate-determining step for the majority of compounds was passive diffusion across the lipid component, which controlled 75-99% of transport. The exception was water, for which transport kinetics was 75% matrix-controlled. In general, permeation across the lipid-filled tissue was more favorable for small lipophilic compounds.

ASTMH POSTER SESSION I

- LYMPHOCYTE ACTIVATION IN VITRO BY ASCARIS SUUM CUTICULAR ANTIGENS.
121 *D.E. Hill, R.H. Fetterer, and J.F. Urban. Helminthic Diseases
Laboratory, LPSI, ARS, USDA, Beltsville, MD.

Ascariasis in humans and swine is a major health problem worldwide. The role of the parasite's cuticle in the pathogenesis of infection is not well understood. The effect of isolated cuticular preparations from distinct developmental stages of A. suum on swine peripheral blood lymphocyte blastogenesis was studied. Mononuclear cells were isolated from swine blood by centrifugation over a cushion of Ficoll-Hypaque. Cells from both non-infected and A. suum infected swine were placed in 96-well culture plates at 2×10^5 cells per well. Cuticular preparations from 2nd, 3rd, and 4th stage larvae were prepared by grinding the larvae in a ground glass homogenizer with 2M urea, 100mM Tris, 3% B-ME, and 30% glycerol, and boiling for 10 min. at 100c. The solution was centrifuged and the pellet (insoluble fraction of the cuticle) was washed 2 times in PBS and digested with trypsin (porcine pancrease) for 24 hrs., then heat inactivated. Cuticles were removed by dissection from 5th stage and adult Ascaris and treated as above. Cuticular preparations were sterile filtered and added to wells with cells at 1, 10, and 30ug protein per well. On day 5 of culture, 0.5uCi of 3H-thymidine was added to each well. Cells were lysed 6 hrs. later and the nuclei collected on glass fiber filters. The nuclear incorporation of 3H-thymidine was determined by liquid scintillation spectrophotometry. Results indicated that cuticular preparations from 3rd stage A. suum specifically stimulated lymphocytes from infected swine but not from non-infected swine. Cuticular preparations from the other stages tested had no stimulatory effect. Cuticular antigens from 3rd stage A. suum may be particularly important in the development of the cellular immune response in Ascaris infections.

- SEROLOGY AS AN INDICATOR OF TAENIA SOLIUM TAPEWORM INFECTIONS
122 IN RURAL VILLAGES OF MEXICO. S.P. Diaz-Camacho, A. Candil Ruiz., M. Beltran Uribe and K. Willms*. Escuela de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, *Instituto de Investigaciones Biomédicas, National University of Mexico.

The epidemiology of Taenia solium tapeworm and the prevalence of serum antibodies to metacestode antigens was studied in two rural villages. Between 25 and 60% of the population of these villages were studied by three serial stool samples, by the methods of Faust, Ritchie and Kato for the detection of tapeworm eggs and other intestinal parasites. Serum samples from each individual were analyzed by the ELISA technique using bladder fluid as antigen. A household census was obtained for each family and in one village it was possible to examine all domestic pigs (72) for the presence of cysticerci under the tongue, however, only one infected pig was found in a household where 2 T. solium tapeworms were diagnosed. The stool analysis demonstrated T. solium in 1.1% (6 cases out of 516 persons) of inhabitants in one village, and in 1.32% (4 cases out of 302 persons) in the second one. Tapeworm infections tended to be clustered in neighboring households in both villages. Overall seropositivity to bladder fluid antigens was between 11 and 12% in both villages. However, seropositivity in family members who had been living with a T. solium tapeworm carrier, was found to be much higher, between 28% and 46%. The difference with the overall seropositivity was highly significant, when tested by chi-square analysis. We interpret the results as proof, that the immune response is a very sensitive indicator of T. solium tapeworms infections in a community, and conclude that widespread application of such a standardized ELISA test would be of help in the rational planning of control measures to prevent cysticercosis in developing countries.

ASTMH POSTER SESSION I

123 EFFECT OF ZINC DEFICIENCY ON THE ESTABLISHMENT AND REPRODUCTION OF AN INTESTINAL NEMATODE IN MICE.

T. Minkus, *M.E. Scott, and K. Koski. McGill University, Montreal
Canada.

Recent interest in nutrition-infection interactions has focussed on the effect of infection on host nutritional status. This study demonstrates the importance of considering the reverse, namely the effect of host nutritional status on parasite biology. The objectives were to investigate the impact of moderate zinc deficiency on establishment and reproduction of the "hookworm" nematode, Heligmosomoides polygyrus, in outbred CD1 mice. Control and zinc-deficient mice were fed ad libitum a semi-purified diet containing 60 and 5 ppm zinc respectively beginning two weeks prior to infection with 100 third-stage larvae. A third pair-fed group was fed the control diet to the level of intake of the zinc-deficient group. In the first experiment, parasite egg production was quantified 14, 21 and 28 days post-infection. Mice were then killed and the parasites were counted. In the second experiment, an anthelmintic-abbreviated vaccination regime was used to stimulate resistance to a challenge infection of 100 third-stage larvae. Egg production and intensities of both primary and challenge infections were monitored. Preliminary results indicate that, during a primary infection, parasite establishment is similar among dietary treatments, but that during a challenge infection, more parasites establish in zinc-deficient mice than in control mice. Secondly, egg production of parasites in zinc-deficient mice is reduced. These data suggest that the impaired immunity associated with zinc deficiency permits higher parasite establishment in challenge infections but that zinc deficiency simultaneously reduces parasite reproduction.

124 PREVALENCE AND RISK FACTORS FOR TAENIA SOLIUM TAENIASIS AND CYSTICERCOSIS IN AN ENDEMIC VILLAGE, MORELOS STATE, MEXICO.

E. Sarti G., P.M. Schantz, A. Plancarte, I. Gutierrez O., V.C.W. Tsang and A. Flisser. Direccion General de Epidemiologia, Secretaria de Salud, Mexico City; Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA; Instituto de Investigaciones Biomedicas, UNAM, Mexico City.

In a village in Mexico where Taenia solium was known to be endemic a census identified 1,750 households with 13,166 residents. A cluster sample of 368 households was selected for demographic, environmental, medical history and diagnostic surveys. All participants were offered a taeniacidal dose of praziquantel. Of 534 pigs examined by tongue inspection 4.3% had cysticerci. Pigs were more likely to be infected if they ran loose or were owned by households whose residents did not use latrines ($p < .01$). Of 1552 human serum samples 11.4% tested positive in the cysticercosis immunoblot assay. At least one seropositive person was resident in 24.2% of sampled households. Histories of chronic headaches and seizures were more common in seropositive than in seronegative persons ($p < .05$). Enterobius vermicularis eggs were identified in 14.9% of 1145 perianal scrapings and Ascaris lumbricoides, Hymenolepis nana, and Iodamoeba butschlii eggs or cysts were found in 6.3%, 3.9% and 9.0%, respectively, of 1531 stool specimens examined by concentration methods. No tapeworm eggs were identified in perianal scrapings or stool specimens, however, five persons passed Taenia sp. proglottids after taking praziquantel and 16 reported having passed proglottids in their feces within the previous 12 months. Preliminary analysis showed that seropositive persons and infected pigs were clustered within households or adjacent households with Taenia carriers.

ASTMH POSTER SESSION I

- 125 Nuclear changes of infected muscle cells correlate with antigen synthesis and secretion by the larva of *Trichinella spiralis*.

D. D. Despommier, W. F. Symmans, T. Edelist and S. Buck. Columbia University, New York, New York.

The larva of *Trichinella spiralis* secretes a protection-inducing antigen into the milieu of its host cell beginning on day 8 post-infection. This antigen localizes to the cytoplasm and nucleoplasm of the host cell, and remains in these sites throughout infection. A unique feature of this intracellular parasitic relationship is the number of new host proteins (eg. collagen) and cell functions (eg. angiogenesis) attendant with the morphogenesis of the muscle cell to the Nurse cell. During transformation, host cell nuclei enlarge and are more numerous than adjacent non-infected fibers. We studied infected host cell nuclei with standard histological techniques on 10- μ m thick serial sections of muscle tissue infected synchronously. Enlargement of nuclei was observed as early as day 5 post-infection ($216 \mu\text{m}^3/\text{nucleus}$; normal muscle nuclei = $100 \mu\text{m}^3$). On days 6, 8, and 10, and 6 months after infection, nuclei had enlarged to 294, 782, 573, and $572 \mu\text{m}^3$, respectively (N=95-100/time point). Six month old Nurse cells contained 46.5 ± 20 nuclei (N=47). A similar length (200 μm) of red fiber has 12-15 nuclei. Thus, *T. spiralis* induces dramatic changes in host nuclei correlating with the secretion of antigen and modulated host genomic expression throughout production and maintenance of the Nurse cell.

- 126 IMMUNODIAGNOSIS OF NEUROCYSTICERCOSIS IN SALIVA

*M. Feldman (1), M.A. Sandoval (2), A. Plancarte (1) and A. Flisser (1). 1. Instituto de Investigaciones Biomedicas, UNAM, 04510 Mexico, D.F., Mexico. 2. Hospital de Especialidades, Centro Medico La Raza, 07000 Mexico, D.F., Mexico.

Cysticercosis, a disease caused by the larvae of *Taenia solium*, has a high prevalence in developing countries. Immunological methods for the detection of antibodies in cerebrospinal fluid (CSF) and serum are reliable for its diagnosis, specially to confirm CT and MR images and for epidemiological studies. Saliva has extensively been used for the detection of antibodies in oral diseases such as caries and only recently it has also been applied to search for specific antibodies against systemic diseases such as AIDS and Hepatitis A.

Here we report the standarization of an enzyme immunoassay using saliva for the diagnosis of human neurocysticercosis, based on the detection of specific IgG antibodies against *Taenia solium* larvae. The cut-off point was defined with the saliva of patients with other neurological pathologies and of healthy individuals. A sensitivity of 76% was obtained when saliva from patients with neurocysticercosis was analyzed as compared to 70% positivity in their serum. The correlation between saliva and serum was 69%. No statistically significant correlation was found between the absorbance values of saliva and the clinical status of the patients, their treatment and the location of the parasites. The use of saliva for diagnosis is a good alternative to serum and CSF because it offers a stress-free, non-invasive sampling procedure. Saliva is simpler to obtain and it is specially useful for field work and epidemiology.

ASTMH POSTER SESSION I

127 INVOLVEMENT OF EXCRETORY-SECRETORY ANTIGENS FROM
JUVENILE ANISAKIS SIMPLEX IN THE IMMUNE-MEDIATED
ADHERENCE OF EOSINOPHILS.

Thomas L. Deardorff^{*1}, Richard E. Jones, and Stephen G. Kayes.
Fishery Research Branch, U.S. Food and Drug Administration,
Dauphin Island, AL., and Department of Structural and Cellular
Biology, University of South Alabama College of Medicine, Mobile,
AL.

We have previously observed that eosinophils were prominent in chronic granulomas around surgically implanted third-stage juveniles of Anisakis simplex in laboratory-infected mice and destruction of the juveniles within the host tissues was initiated by day 14 postinfection. The mechanism for destruction of these juveniles may involve the interaction of eosinophils and excretory-secretory (ES) products released as the worm invades the host's tissues. To test this hypothesis, eosinophils as well as components of the host immune system were harvested by bronchoalveolar lavage from CBA/J mice previously infected with Toxocara canis. The cells were added to cultures of juvenile worms in the presence or absence of immune serum from mice. Adherence of eosinophils occurred only with immune serum. Transmission electron microscopy revealed active degranulation of eosinophils onto the epicuticle of the parasite. Profound damage of the cuticular surface of the juveniles was not evident after 24 hr in vitro.

128 INTESTINAL CAPILLARIASIS: THE SPREAD

John H. Cross, Dept. of Preventive Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD

Intestinal capillariasis was first seen in the Philippines in 1962. In 1965 an epidemic began which continued into 1969-1970. Infections with the parasite, *Capillaria philippinensis*, have continued in Northern Luzon throughout the years with 5-65 patients being seen each year. Over 100 people are known to have died from the infection, most (95) during the first years of the epidemic. Throughout the years most infections have been seen in middle aged men (1329 males, 548 females). Another epidemic occurred in Southern Leyte in the early 1980s and in a survey of some of the affected barrios, 14% of those examined (362) were passing eggs of the parasite. A number of people also died in this epidemic.

The disease was recognized in Thailand in 1972 and since that time more cases and deaths have been reported. Japan reported a case in 1983 and specimens from two other patients have been confirmed as *C. philippinensis*. An Iranian farmer was found suffering from the disease in 1986 and in 1987 an infection was diagnosed in an Egyptian.

Symptoms of borborygmi, abdominal pain and diarrhea are experienced by most of those infected. In the late stage of the disease there is a protein-losing enteropathy, malabsorption and low serum levels of electrolytes and total protein. Death is attributed to heart failure and intercurrent bacterial infections. The diagnosis is made by finding eggs, larvae and adults in the feces. Mebendazole 200 mg bid x 20 days or albendazole 200 mg bid x 10 days are effective anthelmintics. Incomplete treatment can lead to relapse.

Freshwater/brackish water fish serve as intermediate hosts of *C. philippinensis* and persons acquiring infections have eaten raw fish. Fish-eating birds are considered natural hosts, and migratory birds may be responsible for the widespread distribution of the parasitosis.

ASTMH POSTER SESSION I

- 129 IDENTIFICATION OF SPECIES AND STAGE-SPECIFIC ANTIGENS OF PARAGONIMUS WESTERMANI WITH MONOCLONAL ANTIBODIES. *Z. Zihao, S. Yiping and W. F. Piessens. Nanjing Medical College, Nanjing, PRC and Harvard School of Public Health, Boston, MA.

The development of a specific serodiagnostic test for paragonimiasis has been hindered by the extensive antigenic crossreactivity between lung flukes and other trematodes. Here we describe our attempts to develop a "dot-blot" ELISA to detect parasite antigens in sera with monoclonal antibodies (Mabs) to species and stage-specific antigens of P. westermani.

Mabs used in the assays were selected because they reacted with either metacercarial or adult worm antigens, but did not crossreact with extracts of schistosomes (eggs or adult worms of S. japonicum and S. mansoni), Leishmania, B. malayi, human serum or with phosphorycholine determinants. When two microliter of sample is spotted onto nitrocellulose paper, 0.05-1 ng of P. westermani extract (from either the metacercarial or the adult worm stage) diluted in normal human serum can be detected with the Mabs. The sensitivity and specificity of this antigen-detection assay is currently being tested with samples collected in the field.

- 130 ISOLATION AND CHARACTERIZATION OF MOUSE EOSINOPHIL SPECIFIC GRANULE PROTEINS FOLLOWING Toxocara canis INFECTION. F. J. Herndon and S. G. Kayes. Department of Microbiology and Immunology, and Structural and Cellular Biology, University of South Alabama College of Medicine. Mobile, AL.

Immunological reagents which recognize eosinophil specific granule proteins of humans and several other mammals have been useful tools in dissection of eosinophil function in host defense and disease. Antibodies to mouse eosinophil specific granule proteins, which would allow further examination of eosinophil function in inbred and immunologically characterized strains of mice, do not presently exist. Utilizing the method of bronchoalveolar lavage of CBA/J female mice infected with second-stage larvae of Toxocara canis, populations of eosinophils > than 90% pure and averaging 6.5×10^6 cells/mouse were obtained. From this cell population, eosinophil granules were isolated (as monitored by electron microscopy), solubilized, and analyzed by SDS-PAGE. Seven proteins with apparent molecular weights from 15 to 66 kilodaltons were observed. To produce polyclonal antibody preparations, these proteins are currently being eluted and used to immunize rabbits. Possession of these reagents will facilitate immunocytochemical localization and quantification of these specific granule proteins in mouse tissues and body fluids and permit physiologic studies of protein function and toxicity. (Supported by Grant AI19968 from NIH and 870034 from the American Heart Association, Alabama Affiliate)

ASTMH POSTER SESSION I

- 131 SERINE PROTEASES FROM TWO INVASIVE ASCARIDOID NEMATODES.
Jamie Deneris and Judy A. Sakanari. Department of Pathology,
University of California, San Francisco, CA.

Serine proteases have been implicated in the pathogenesis of a number of infectious diseases. Aside from their role in the physiology and metabolism of organisms, parasite proteases may facilitate invasion of host tissue, metabolism of host proteins, and evasion of the host immune response. Anisakiasis is a human disease caused by the ingestion of the larval nematode Anisakis sp. found in raw seafood dishes such as sushi and sashimi. This parasite can be invasive and penetrate the wall of the stomach or intestine. We have found that secretions of tissue-invasive larvae contain a trypsin-like serine protease that may facilitate the invasion of host tissue. Based upon the characterization of this enzyme by substrate gel electrophoresis, peptide substrate assays, and inhibition studies, we hypothesized that it was structurally related to the trypsin family of serine proteases and not the subtilisin family. Serine protease gene fragments were isolated from genomic DNA of Anisakis simplex by using degenerate oligonucleotide primers and polymerase chain reaction. Primers were designed based on the consensus sequence of amino acids flanking the active site serine and histidine residues of eukaryotic serine proteases. Serine protease gene fragments from this parasite were sequenced. One of these, a 470 bp fragment, has high identity with mammalian trypsinogens. Results of a Southern blot of Ascaris suum probed with the 470 bp Anisakis fragment showed that a similar gene is present in this closely related nematode.

- 132 SAFETY AND IMMUNOGENICITY OF A NEW CHIKUNGUNYA VIRUS VACCINE:
DOULBE-BLIND, PLACEBO-CONTROLLED HUMAN TRIAL.

*F.J. Malinoski, T. Ksiazek, H. Ramsburg, H.W. Lupton, G.F. Meadors., US Army
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Chikungunya (CHIK) is a mosquito-borne viral disease associated with high attack rates. Illness is characterized by fever, rash, acute arthralgia or arthritis lasting 1 to 2 weeks, and chronic arthritis in 2 to 12 % of patients. CHIK is a member of the alphavirus genus of the family *Togaviridae*.

A live, attenuated vaccine was administered in a double-blind and placebo controlled trial to healthy active duty soldiers.

Minor clinical abnormalities were observed in both groups and included arthralgias, headaches, myalgias, and skin flushing. There was no difference between the groups in terms of rates of these minor reactions, although there were more total days of arthralgias in the vaccine group. Vaccine virus was isolated from twenty-one serum samples from 12 vaccine recipients between days 2 and 9 after inoculation.

All 30 vaccine recipients had measurable immune responses as determined by plaque-reduction neutralization titers and IgM ELISA, whereas none of the control population seroconverted. PRNT-80 titers and IgM levels in the vaccine recipients peaked concurrently between days 14 and 35. Cross reactivity of these sera with other CHIK strains and other members of the alphavirus genus are presented.

On the basis of these data, the vaccine appears to be safe and immunogenic and the potential use of the vaccine in endemic areas of the world is discussed.

ASTMH POSTER SESSION I

- 133 VENEZUELAN EQUINE ENCEPHALITIS (VEE) SPECIFIC IgG SUBCLASS RESPONSES FOLLOWING IMMUNIZATION WITH TC-83 AND C-84 VACCINE. *R.J.M. Engler, C.J. Peters, P.B. Jahrling, M. Pedrotti, J. Mangiafico, and C. Reimer. Walter Reed Army Institute of Research, Washington, D.C.; U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD; and Centers for Disease Control, Atlanta, GA.

Two vaccines are currently available for active immunization against VEE infection: TC-83, a live-attenuated vaccine, and C-84, a formalin-inactivated vaccine derived from the TC-83 strain. Viral neutralization assays (VNA) have been utilized to assess immunity and need for booster immunization. We developed an enzyme-linked immunoadsorbent assay (ELISA) for the measurement of VEE-specific IgG subclasses in order to determine the predominant response to the VEE vaccines and correlate these responses to the VNA. Sera prior to and 3 to 4 weeks following either TC-83 vaccination alone (20 laboratory workers) or following C-84 booster immunization (19 laboratory workers who had originally received TC-83) were collected and frozen (-20°C) until ready for study. Both the TC-83 and C-84 groups were assayed for VEE specific total G, G1, G2, G3 and G4 in parallel with the 80% plaque reduction viral neutralization. Both groups demonstrated predominantly VEE-G1 and/or G3 booster responses (>4 fold titer increase), correlating to the VNA titer with a P value <0.01 for G3 and <0.05 for G1. Thus, VEE specific in vitro neutralization appears to correlate most specifically with the G1 and G3 subclass response and both types of vaccine generate this response.

- 134 TITLE: FOURTEEN YEARS OF EXPERIENCE WITH INACTIVATED EASTERN EQUINE ENCEPHALITIS VACCINE. *N. A. Popovic, D. D. Oland, and J. A. Mangiafico. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

The Eastern equine encephalitis vaccine (EEE) was produced from the PE-6 strain of the virus grown in primary chick embryo cells and later inactivated with formalin. The vaccine is used at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) to immunize employees at potential risk of exposure to virulent EEE virus. The EEE vaccine was administered under an Investigational New Drug Protocol (IND-266). For purposes of this study, data are reported on 428 individuals vaccinated from 1975 to 1989. All vaccinees had negative prevaccination plaque-reduction neutralization (PRN_{80}) titers. The primary injection series consisted of 2 subcutaneous 1-ml doses. Ninety-four percent of vaccinees who received the 2nd injection 29-35 days after the initial injection developed neutralizing antibodies (PRN_{80} 1:10). Optimal time for evaluation of the neutralizing titer was 21-28 days after completion of the primary series. A PRN_{80} titer of 1:40, presently considered to be a minimal protective level, was obtained in 76.9% of the vaccinees. No systemic reactions were associated with primary vaccination. Erythema at the injection site was noted in 4 vaccinees. This resolved without complications. These data indicate that the EEE vaccine as administered in the study protocol is safe and immunogenic.

ASTMH POSTER SESSION I

135 EVALUATION OF THE SAFETY & IMMUNOGENICITY OF AN INACTIVATED JAPANESE ENCEPHALITIS VACCINE IN U.S. LABORATORY PERSONNEL.

M.A. Ussery*, N.A. Popovic, D. Oland, and J. Magnifico. U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

One hundred thirty four individuals at USAMRIID and sixty individuals at Southern Research Institute, Birmingham, AL (SoRI) were immunized with three subcutaneous 1 ml doses of Japanese encephalitis (JE) vaccine (developed by the Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan) on days 0, 7 and 14. The JE vaccine was administered under an Investigational New Drug Protocol (IND-1824). Each vaccinee had a negative pre-vaccination titer (PRNT₅₀). The initial post-vaccination titers were obtained in the majority of vaccinees (114/134) between 13 & 30 days after completion of the third injection. Eighty nine % of the USAMRIID and 58% of the SoRI vaccinees developed a PRNT₅₀ of $\geq 1:10$, with a median titer of 1:40 and a geometric mean titer of 31.6. Twenty one vaccinees complained of mild local discomfort and erythema at the injection site, usually noted 24 to 48 hr after each injection. Seventeen vaccinees related mild systemic complaints, including headache, myalgia and malaise. These symptoms usually occurred within 48 hr after the first two immunizations, but persisted in several vaccinees up to 6 days after the 3rd immunization. There were no cases of allergic or anaphylactic reactions or vaccine associated encephalitis in the USAMRIID vaccinees and one case of allergic reaction after the first immunization of a SoRI employee. At SoRI, 14 vaccinees who had titers of less than 1:10 were boosted with one immunization 3 to 7 months later; 11/14 (79%) developed post-boost titers of ≥ 10 . These data indicate that the JE vaccine as administered in the study protocol, is both safe & immunogenic.

136 PROTECTIVE EFFICACIES IN RHESUS MONKEYS OF CANDID 1 STRAIN JUNIN VIRUS (JV) AND TACARIBE VIRUS AGAINST AEROSOL CHALLENGE WITH VIRULENT JV.

*R.H. Kenyon, K. McKee, Jr., J. Barrera Oro, D. Ragland, C. Crabbs, and Y. Higgins. USAMRIID, Ft. Detrick, Frederick, MD.

Candid 1 (C-1) JV vaccine for Argentine hemorrhagic fever protects against parenteral virulent JV and Machupo (MAC) virus challenge in animal models for the disease. Tacaribe virus (TAC), a related arenavirus, has been reported effective in inducing protection against parenteral JV challenge in virulent JV animal model systems. Efficacy against aerosol challenge, the presumed natural route of infection, has not been established with either virus. We inoculated 4 monkeys IM with C-1 JV and 4 with TAC. Serum viremia was detected in 3/4 C-1 vaccinees and in 1/4 TAC vaccinees by standard plaque assays, and in 4/4 C-1 animals by co-cultivation of peripheral mononuclear cells. All C-1 vaccinees developed high neutralization titers to JV (GMT=1:4096). In contrast, all TAC vaccinees developed high titers to TAC, and 3/4 developed a low titer to JV (GMT=36). By lymphocyte transformation assays, 4/4 C-1 vaccinees responded to JV, 4/4 responded to MAC virus, and 2/4 responded to TAC virus. All 4 TAC vaccinees responded to MAC virus as well as TAC virus, and 2 responded to JV. Six months after vaccination, all vaccinees and 3 unvaccinated controls were challenged by a small-particle aerosol of virulent JV. No vaccinees showed signs of illness after challenge, but the 3 controls became acutely ill and died. Controls were viremic on day 7 and remained viremic until death. Serum viremia was not detected among C-1 vaccinees at any time after challenge, while 1 TAC monkey showed a low-level viremia on day 5. All vaccinees were killed at 60 days after challenge; microscopic examination of the tissues is in progress. Both C-1 and TAC viruses afforded protection to an aerosol challenge with virulent JV.

ASTMH POSTER SESSION I

137 INACTIVATED HEPATITIS A VACCINE (CLF STRAIN) IN HEALTHY ADULT VOLUNTEERS. *M Sjogren, C Hoke, L Binn, J Sanchez, L Lyde, E D'Hondt, J Boscia, W Bancroft. Walter Reed Army Institute of Research, Washington, DC and Smith Kline Beckman, Philadelphia, PA and Belgium.

Prior work with an inactivated, non purified, low concentration HAV antigen vaccine (strain HM-175) resulted in detectable anti-HAV in 86% of 42 volunteers. To test if a purified, alum adjuvanted, concentrated inactivated HAV vaccine CLF strain was safe and immunogenic in man, we administered the CLF-derived HAV vaccine to 10 volunteers at 0, 1 and 6 months interval. Local and systemic side effects and serum ALT were monitored after each immunization. Total and IgM anti-HAV were evaluated using commercial assays (Abbott Laboratories) and neutralizing antibody by RIFIT. To date all volunteers have received the first two injections, and sera have been tested at time 0 and 90 days after the initial immunization.

	Time 0	Time 90 days
Mean Total Anti-HAV(Range)	2.8% (-11%-7.5%)	40% (21%-60)
Neutralizing antibody (Titer)	0/10	10/10 (1:10-1:40)
IgM anti-HAV	0/10	0/10
Mean ALT (Range: 2-50 IU/L)	19.5 (13-33)	18.8 (11-33)

No systemic side effects were observed. Sore arms were reported following 8 of 20 immunizations, described as mild in 4 and moderate in 4. No muscle aches were reported. Mild headache was reported by 3 volunteers. One man had malaise following each immunization, it was short lived and without significant illness. No cases of hepatitis were observed. These preliminary data strongly suggest that this hepatitis A vaccine (CLF strain) is safe and immunogenic.

138 LOW-DOSE INTRADERMAL RECOMBINANT HEPATITIS B VACCINE. J. BRYAN*, M. IQBAL, M. SJOGREN, A. AHMED, A. RAUF, S. NABI, B. COX, A. MORTON, J. SHUCK, P. MACARTHY, I. MALIK, P. PERINE, L. LEGTERS. Uniformed Services University of the Health Sciences, Bethesda, Md.; Army Medical College, Rawalpindi, Pakistan; Walter Reed Army Inst. of Research, Washington, D.C.

We investigated the immunogenicity and reactogenicity of low dose, recombinant DNA and plasma-derived hepatitis B vaccines in a prospective, double blind, controlled trial. 154 volunteers were randomized to receive either Recombivax HB®, 10 µg in 1 ml IM, Recombivax HB® 1 µg in 0.1 ml ID, or plasma derived Heptavax B®, 2 µg in 0.1 ml ID at 0, 30, and 150 days. Results of anti-HBs levels at day 270 and side effects are as follows:

	Recombivax HB®				Heptavax B®
	10 µg n=47	P	1 µg n=49	P	2 µg n=49
Mean anti-HBs (mIU)	254.8	<0.05	81.6	<0.05	192.3
No. (%) ≤ 9.9 mIU	3(92)	0.01	11 (22)	0.05	5 (10)
Local reactions (%)					
erythema ≥ 5 mm	7	<0.001	80	NS	82
induration ≥ 5 mm	4	<0.001	67	NS	74
pruritus	6	NS	16	NS	10
painful extremity	17	NS	10	NS	6

Conclusions: 1 µg of intradermal Recombivax is inferior to 2 µg of ID Heptavax and to 10 µg of Recombivax IM. 2 µg ID Heptavax is not significantly less immunogenic than 10 µg of Recombivax at 1/10 the cost with acceptable local reactions.

ASTMH POSTER SESSION I

139 EFFICACY OF A MASS RECOMBINANT HEPATITIS B VACCINATION PROGRAM IN PRIMARY SCHOOL CHILDREN INFECTED WITH *S. MANSONI*.

*S.Bassily, M.F.Abdel Wahab, T.Strickland, G.Esmat, S.Narooz, N.El-Masry, N.Constantine and E.Medhat. U.S. NAMRU-3, Cairo, Egypt; Cairo University, Cairo, Egypt and University of Maryland School of Medicine, Baltimore, MD.

After parents consent, 508 children of a primary school in Defra village, Egypt, were included in the study. Ages ranged from 6-12 years and male/female ratio was 3:2. All were examined clinically and tested for *S.mansoni*, *S.haematobium*, ALT and hepatitis B markers (HBsAg, anti-HBs and anti-HBc) using standard techniques. Abdominal sonograms were done for 104 cases. Four hundred and forty two students (119 of whom were positive for *S.mansoni*), negative for hepatitis markers and with ALT <50 units were vaccinated with three 5 µg doses of Recombivax (MS&D) given I.M. in the deltoid region at 0, 1 and 6 months. After one year all students were re-examined, sonograms repeated, stool and urine tested for schistosomiasis and sera tested for hepatitis B markers. A quantitative ELISA test was used for anti-HBs levels. Children positive for schistosomiasis were treated with praziquantel given in a single oral dose of 40 mg/kg/body wt. The initial evaluation showed prevalence rates of 47.5% for *S.mansoni*, 0.8% for *S.haematobium*, 3.5% for HBsAg, 15.2% for hepatitis B markers. All were significantly higher in males compared to females. Vaccine was well tolerated (only 16 had local soreness at site of injection). All vaccinees, except two, sero-converted (rate 99.5%) and only 3 others (0.7%) had anti-HBs levels below the protective levels (<10 MIU/ml). Twenty had levels of 10-100 MIU/ml. The other 417 had a mean level of 7379 MIU/ml. HBsAg was found in 2 sera and anti-HBc in 15. The study shows that a five µg dose of Recombivax is safe and immunogenic to children 6-12 years old whether they have active *S.mansoni* or not. Prolonged follow-up is needed for both groups.

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140 ANTIGENIC ANALYSIS OF A GLOBAL COLLECTION OF HANTAVIRUS STRAINS. Y.K. Chu, S.E. Hasty, C.S. Schmaljohn, C.A.

Rossi, J.A. LeDuc and J.M. Dalrymple. Department of Viral Biology, Virology Division and Department of Epidemiology, Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland, 21701-5011.

Hantaan is the prototype virus of the *Hantavirus* genus of the family Bunyaviridae. Many hantaviruses are etiologic agents of human diseases which are collectively described as hemorrhagic fever with renal syndrome. A broad spectrum of human disease severity is associated with different hantaviruses ranging from Hantaan virus induced severe disease such as Korean or epidemic hemorrhagic fever to a milder syndrome of nephropathia epidemica caused by Puumala virus. Some members of the genus, such as the United States isolate Prospect Hill virus, have not been associated with any human disease. An estimation of the amount of antigenic variation within the genus becomes important for vaccine development and the generation of improved diagnostic reagents. We have attempted the serological comparison of greater than thirty different virus isolates and strains from representative geographic areas and ecologic habitats of the world. Each of these viruses were examined using the techniques of fluorescent antibody, ELISA, plaque reduction neutralization, and hemagglutination inhibition with infected laboratory rat and rabbit sera as standard antisera as well as a battery of monoclonal antibodies. Immune precipitation of radiolabeled virus proteins was used to identify major structural antigens. More than five major serogroups have been identified on the basis of serological reactivity.

ASTMH POSTER SESSION I

141 CLONING AND SEQUENCE ANALYSIS OF THE L GENOME SEGMENTS OF TWO VIRUSES IN THE *HANTAVIRUS* GENUS OF BUNYAVIRIDAE

H. F. LaPenotiere and C. S. Schmaljohn. United States Army Medical Research Institute of Infectious Diseases. Fort Detrick, Frederick, MD

The prototype member of the *Hantavirus* genus of Bunyaviridae, is the etiologic agent of Korean hemorrhagic fever, Hantaan virus. Hantaviruses have been associated with a number of clinically similar diseases collectively termed hemorrhagic fever with renal syndrome (HFRS). Like other viruses in the family, hantaviruses have a three-segmented, single-stranded RNA genome. Previous studies have focused on the small (S) and medium (M) segments of the hantaviral genome, which encode the nucleocapsid and envelope glycoproteins, respectively. The large (L) genome segment is presumed to encode the viral transcriptase protein, necessary to generate message-sense RNA from genome-sense RNA. In order to better understand the replication processes of hantaviruses, to identify gene regions conserved within the genus, and to define properties of transcription which might be targets for antiviral intervention, we examined the L segments of two hantaviruses, Hantaan and SR-11 viruses. cDNA libraries were prepared from purified virion RNA, and were cloned into the Lambda-Zap II vector (Stratagene). Clones that appear to represent the entire L genome segments of both viruses have been identified. Sequence analysis of cDNA clones is in progress, and, once completed, will serve as the basis for comparative analyses of this presumed polymerase gene.

142 CLONING AND SEQUENCE ANALYSIS OF THE M AND S GENOME SEGMENTS OF THE *HANTAVIRUS*, SR-11 VIRUS.

L. Iacono-Connors, H. LaPenotiere, J. Arikawa and C. S. Schmaljohn. United States Army Medical Research Institute of Infectious Diseases. Fort Detrick, Frederick, MD

Hemorrhagic fever with renal syndrome (HFRS) is caused by certain viruses in the *Hantavirus* genus of Bunyaviridae. The prototype virus of the genus, Hantaan virus, was isolated from *Apodemus* mice, and is the etiologic agent of Korean hemorrhagic fever. A less severe form of HFRS is associated with rat-borne hantavirus infection. To examine the usefulness of a recombinant vaccine prepared by using Hantaan viral cDNA against rat-type hantaviruses, and to define conserved regions that might be useful in diagnosis or immunity, we cloned and sequenced the M and S genome segments of Sapporo rat virus (SR-11) and compared them to those of Hantaan virus. Synthetic oligonucleotide probes corresponding to conserved or unique gene regions were used to differentiate Hantaan and SR-11 RNAs by Northern blot analysis. This technique may have application for diagnosis of HFRS in regions where both types of hantaviruses are circulating. Amino-terminal sequence analysis of purified G1 and G2 revealed conserved sequences between Hantaan and SR-11, suggesting that the electrophoretic migration differences between the G1 proteins of these viruses may be due to modifications of mature proteins rather than true molecular weight differences. Approximately 75% of the amino acids comprising G1 and G2 were conserved between the two viruses, demonstrating a molecular basis for the observed serologic cross-reactivity.

ASTMH POSTER SESSION I

143 ANTIGENIC ANALYSIS OF THE STRUCTURAL PROTEINS OF CRIMEAN-CONGO
HEMORRHAGIC FEVER VIRUS

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A panel of 106 independently-derived monoclonal antibodies has been generated to the structural proteins of the 10200 strain of CCHF virus. These antibodies are being utilized to define the antigenic relationships among CCHF viral strains as well as other Nairoviruses, and to identify neutralizing and protective epitopes. The results of these studies have indicated that epitopes on the 50 kDa nucleocapsid protein are highly conserved among African and Asian CCHF strains, but distinct from those found on heterologous Nairoviruses. In contrast, epitopes on the 80 kDa G1 and 36 kDa G2 glycoproteins have shown more heterogeneity among various CCHF strains, and were often expressed on other Nairoviruses. Virus neutralizing capacity, as measured in plaque reduction assays (PRNT), was demonstrable only with anti-G1 monoclonal antibodies with 18/36 anti-G1 monoclonal antibodies and 0/15 anti-G2 monoclonal antibodies showing significant neutralization activities. However, passive immunization studies, carried out in infant mice, have shown a lack of correlation between *in vitro* neutralization and *in vivo* protection. Efficient protection has been achieved with neutralizing anti-G1 as well as non-neutralizing anti-G2 antibodies, and several antibodies with high PRNT titers have provided no protection.

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T-LYMPHOCYTE RESPONSES TO HUMAN PAPILLOMA VIRUS PROTEINS.

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Human T-lymphocyte determinants have been identified in the major structural antigen (L1), and in an internal, transformation associated protein (E6) from a human papilloma virus strain (HPV-16) believed to be associated with cervical cancer. They were defined with the aid of synthetic peptides chosen on the basis of structural similarities with previously defined T-lymphocyte determinants. This strategy was extremely useful for studying human responses to papilloma virus because of the absence of any permissive culture system for HPV and the corresponding difficulties of obtaining HPV infected cells or HPV antigens with which to probe for immune responses.

Lymphoproliferative assays using PBMC from both woman with cervical neoplasia (stages I-III) and healthy donors were performed in order to determine responsiveness to peptides. Proliferation to some peptides of PBMC from individual patients was observed. Surprisingly lymphocytes from healthy donors with no apparent HPV associated disease, also proliferated when incubated with the papilloma peptides. These results suggest that papilloma virus infection may be more widespread than previously suspected. Interleukin 2-dependent peptide-specific T-lymphocyte lines were established and the HLA restriction of the peptide recognition was defined.

ASTMH POSTER SESSION I

145 A DENGUE VIRUS PLAQUE ASSAY UTILIZING THE C6/36 Aedes albopictus MOSQUITO CELL LINE.

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Plaque assays of dengue viruses have historically been performed using vertebrate cell lines; most notably the monkey kidney cell line LLC-MK2. Since infectious dengue virus titers estimated by mosquito inoculation frequently exceed titers obtained by direct plaque assay on vertebrate cells, mosquito cells were investigated as a cell substrate for comparative plaque assays. The *Aedes albopictus* C6/36 cell line was employed using cells propagated at 28°C as an adherent cell monolayer and an agarose overlay of infected cells incubated at 35°C. Numerous isolates and strains of each of the four dengue serotypes were evaluated using this plaque assay technique which was extended to examine its applicability to numerous other flaviviruses as well as selected alphaviruses and members of Bunyaviridae. Although certain virus strains exhibited five- to ten-fold higher titers by mosquito cell plaque assay, others were equivalent or lower than those from vertebrate cell based assays. Interestingly, plaque morphology appeared to correlate with dengue virus virulence in that viruses with "clear" plaques were generally associated with strains of increased human and/or monkey virulence. This mosquito cell plaque assay has been routinely employed for monitoring dengue viremias in experimentally infected monkeys and human vaccine volunteers and has consistently yielded higher dengue titers than corresponding LLC-MK2 assays. Isolates from human infections have been quantified by assaying directly from patient sera. The numerous advantages of this assay method will be detailed.

146 DEVELOPMENT OF A DOT-IMMUNOBINDING ASSAY FOR DETECTING IgM ANTIBODIES TO DENGUE IN ACUTE AND CONVALESCENT SERA.

J.J. Oprandy¹, P.L. Summers², D.J. Gubler³, C.G. Hayes⁴, D. Sieckmann¹ and R. Wistar Jr.¹ ¹Naval Medical Research Institute, Bethesda, MD; ²Walter Reed Army Institute of Research, Washington, D.C.; ³Centers for Disease Control, San Juan, PR and ⁴Naval Medical Research Unit No. 2, Manila, PI.

A dot-immunobinding assay (DotIA) utilizing a novel membrane, polyvinylidene difluoride (PVDF) is described. This assay was developed for the rapid detection of IgM antibodies to dengue fever (DEN) virus. A monoclonal antibody to human IgM was bound to PVDF for antibody capture. Membranes were incubated with sera, strips were then washed and incubated with DEN antigen. Specific antigen-antibody complexes were detected with an enzyme labeled monoclonal antibody to DEN. Development with substrate yielded a colored insoluble product which was quantified by densitometry. Total assay time was 1.5 h.

Acute and convalescent sera from virologically confirmed dengue patients were assayed. Dengue specific IgM antibodies were detected in twenty-nine of fifty-seven acute sera (51%) and in forty of fifty-seven (70%) convalescent sera tested for all four subtypes combined. DEN 2 patient convalescent sera were positive for IgM at a rate of 82.3% (14/17). Results are discussed with antigen detection and ELISA data. Recent refinements of this assay system are also presented.

ASTMH POSTER SESSION I

147 USE OF FLAVIVIRUS-E. COLI FUSION PROTEINS AS DIAGNOSTIC REAGENTS TO DISTINGUISH INFECTIONS BY DIFFERENT FLAVIVIRUSES.

WuFang Fan, Robert E. Shope, Benedito Fonseca, Martin U. Zuegel, Maurille J. Fournier, Thomas L. Mason, and Peter W. Mason. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT, and Department of Biochemistry, University of Massachusetts, Amherst, MA.

We have recently shown that *E. coli* fusion proteins containing amino acid residues 300 to 400 of the Japanese encephalitis virus (JE) E protein are recognized by anti-JE monoclonal antibodies (MAbs) and the antibodies present in mice hyperimmunized with JE (Mason *et al.*, *J. Gen. Virol.*, *In Press*). A similar region of the dengue type 1 virus (DEN1) E protein has been expressed as a fusion protein in *E. coli*. The DEN1 fusion protein reacts with anti-DEN1 MAbs and with antibodies present in mice hyperimmunized with DEN1. Comparisons of the reactivity of these two recombinant antigens with murine immune ascitic fluid (MIAF) generated against several different flaviviruses (including: JE, DEN1, DEN2, DEN3, DEN4, yellow fever, West Nile, Kunjin, St. Louis encephalitis, and Murray Valley encephalitis) showed strong positive reactions with the homologous MIAFs (JE, or DEN1), and low reactivities with the remaining MIAFs. Preliminary analysis of human sera from individuals infected with DEN1 or immunized with an inactivated JE vaccine, showed the expected positive reaction with the appropriate recombinant antigen. We are currently working on producing analogous fusion proteins for DEN2, DEN3, and DEN4. These antigens have the potential for identifying specific antibodies in individuals who have been exposed to more than one flavivirus.

148 A STUDY OF ANTIBODY RESPONSE TO DENGUE NS1 BY WESTERN BLOT. G. Kuno,* A.V. Vorndam, D.J. Gubler, and I. Gómez. Dengue Branch, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, GPO Box 4532, San Juan, Puerto Rico 00936

As an alternative to attenuated vaccines for dengue, subunit vaccines composed of a nonstructural protein (NS1) are being developed in other laboratories. Before their trials in humans, it is important to examine immune responses in natural infections. We first studied the dynamics of immune responses in 2 cases of primary and 3 cases of secondary infections from which multiple specimens were available. Anti-NS1 antibody was not detectable in the primary infections but was detectable in 2 of the 3 secondary infections in the specimens collected on day 15 after onset or vaccination. Detection of the antibody was often associated with high HI antibody titer of specimen. In these 2 cases titers to NS1 were lower than those to E-protein. Three hundred and one serum specimens from confirmed primary and secondary dengue infections collected in Puerto Rico and in Indonesia were examined. It was found that in primary infections the antibody was detectable only in a small proportion of convalescent specimens (8% in Puerto Rico and 0% in Indonesia). In the acute phase of the secondary infections, the difference in proportions of positive specimens between Puerto Rico (14.7%) and Indonesia (47.7% for classic fever; 62.5% for DHF) was statistically significant. Apparently, in acute phase of secondary infections Indonesians responded immunologically more intensely to NS1 than Puerto Ricans. In the convalescent phase, the difference between Puerto Rico (58%) and Indonesia (84.6%) was only marginally significant, while that between classic fever (82.6%) and DHF (84.6%) in Indonesia was insignificant.

ASTMH POSTER SESSION I

- 149 OPTIMIZATION AND APPLICATION TO HUMAN DIAGNOSTIC TESTING OF HANTAAAN VIRAL ANTIGENS FROM INFECTED CELL CULTURE CELLS. *F.R. Bethke, C.A. Rossi, S. Sebero, J.W. LeDuc, and T.G. Ksiazek. USAMRIID, Ft. Detrick, Frederick, MD.

Hantaan viral antigens were obtained by detergent extraction from E6 cells infected with prototype (76-118) virus. The viral extract efficiently coated wells of polyvinyl chloride microtiter plates which were used in enzyme immunosorbent assays for determination of IgG in human sera obtained from well-characterized cases of Korean hemorrhagic fever. In these patients, the Hantaan virus-specific IgG response, although lagging behind that of Hantaan-specific IgM, rose rapidly within 2 weeks of onset to significant titers ($>12,800$), with optical density values greater than 1.5 at an initial dilution of 1:100. Both titers and optical density values remained high for sera of other patients more than 1 year after onset of disease.

Thus, this Hantaan viral antigen, which can be efficiently prepared, inactivated, and adsorbed to the wells of microtiter plates, provides a practical means of rapidly screening for past infection with Hantaan virus. Similar antigens have also been prepared with the related Hantaviruses, Puumala and Seoul; and we are determining the suitability of these antigens for serological screening for evidence of past infections.

- 150 DIAGNOSTIC POTENTIAL OF A BACULOVIRUS-EXPRESSED, NUCLEOCAPSID PROTEIN FOR HANTAVIRUSES. *C.A. Rossi, C.S. Schmaljohn, and J.W. LeDuc. USAMRIID, Ft. Detrick, Frederick, MD.

A non-infectious, baculovirus-expressed recombinant protein, analogous to the nucleocapsid of Hantaan (HTN) virus and standard Vero E-6 cell culture-prepared HTN antigen, were evaluated in enzyme-linked immunosorbent assays (ELISA) to detect IgG antibodies to various strains of hantaviruses. Hantaan immunofluorescent antibody (IFA)-positive and -negative rat and human sera, confirmed by the plaque-reduction neutralization tests (PRNT), were examined. Results obtained by the IFA test, ELISA (using both antigen preparations) and PRNT were compared. Sera with IFA titers greater than 1:128 showed a high degree of correlation with both ELISA antigens and the PRNT. ELISA tests utilizing the recombinant protein were found to be as sensitive as the PRNT in detecting antibodies to HTN, Seoul, and Purogia viruses. Recombinant protein was found to be less sensitive in detecting Puumala virus antibodies, as has been observed with the standard ELISA antigen preparation. Our results suggest that the IgG ELISA utilizing the HTN recombinant nucleocapsid protein is a useful alternative to the IFA for routine screening for HTN antibodies. This test is sensitive, easy to perform, rapid, and requires very small amounts of serum. Furthermore, the antigen source is a non-infectious recombinant and, therefore, safe to prepare and use.

ASTMH POSTER SESSION I

151 RAPID DETECTION OF HEPATITIS A VIRUS IGM ANTIBODIES BY A SOLID PHASE ANTIBODY CAPTURE HEMADSORPTION ASSAY.

*P.L. Summers, D.R. Dubois, W.H. Cohen, P.O. Macarthy, L.H. Binn, M.H. Sjogren, R. Snitbhan, B.L. Innis, and K.H. Eckels. Walter Reed Army Institute of Research, Washington, DC, and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

A hemagglutination-inhibition (HAI) test for the detection of antibodies to hepatitis A virus (HAV) has recently been developed. Although the HAI test is a simple and sensitive test to measure HAV antibodies, it does not distinguish between recent and past infections which is necessary for serodiagnosis. Therefore, the HAV HAI test was modified for the specific detection of HAV IgM antibodies using antibody capture methodology. Optimal parameters and test conditions for the solid phase antibody capture hemadsorption (SPACH) test were determined. A panel of sera collected from known cases of HAV, hepatitis B virus (HBV) and non A, non B hepatitis (HNANB) were evaluated by SPACH and other serological tests for hepatitis. Preliminary testing of several of the sera indicated that the SPACH was specific and identified IgM in sera separated on sucrose gradients. Serum specimens collected up to 188 days post illness were positive for IgM by SPACH testing. The geometric mean SPACH titer for the acute sera was 145,000. IgM antibodies (titers <100) were not found in sera from HBV or HNANB cases even though many of these had evidence of a past exposure to HAV as indicated by HAV antibodies. The SPACH test was compared to a commercially-available radioimmunoassay and was found to be equally sensitive for detection of specific IgM antibodies. Our results indicate that the SPACH test is a sensitive and specific assay for the rapid diagnosis of HAV.

152 PREPARATION OF NONINFECTIOUS HEPATITIS A VIRUS HEMAGGLUTININ FOR DETECTING HEMAGGLUTINATION INHIBITION ANTIBODIES.

*D.R. Dubois, L.N. Binn, P.L. Summers, R.L. Timchak, D.A. Barvir, R.H. Marchwicki, and K.H. Eckels. Walter Reed Army Institute of Research, Washington, DC

Hepatitis A virus (HAV) harvested from infected MRC-5 cells can hemagglutinate various species of erythrocytes at acidic pH. Further studies revealed that the majority of hemagglutinating (HA) activity in MRC-5 cells was cell-associated. A simplified procedure for preparing HAV hemagglutinin consisted of collecting infected cells in 10 ml of 6.7 mM phosphate-buffered saline followed by three cycles of freeze-thawing and sonication. The clarified fluids were passed through a 0.45 μ m membrane filter and stored at 4°C. The analysis of HA antigen by rate-zonal sucrose gradient centrifugation indicated that the majority of HA activity was associated with infectious virus. Complete inactivation of HAV with 0.03% beta-propiolactone (BPL) did not affect HA activity, while inactivation with 0.05% formalin caused a 16-fold reduction in titer. When BPL-treated hemagglutinin was compared to infectious hemagglutinin in the hemagglutination inhibition (HAI) test, there was no difference in HAI antibody titers. A simple method of producing noninfectious HAV hemagglutinin is described that may be used for diagnostic, epidemiological and vaccine evaluation.

ASTMH POSTER SESSION I

- 153 CRITERIA FOR DETECTION AND QUANTIFICATION OF ANTIBODY-DEPENDENT
ENHANCEMENT OF INFECTION WITH FLAVIVIRUSES IN VITRO. D.M. Morens &
S.B. Halstead. Department of Tropical Medicine, University of Hawaii,
Rockefeller Foundation, New York.

Criteria for detecting and quantifying antibody-dependent enhancement in vitro have never been universally accepted for flaviviruses, or for other viruses that appear to exhibit antibody-enhanced infection, including the human immunodeficiency virus (HIV). Recently several reports of possible infection enhancement with HIV have raised calls for cessation of vaccine trials already underway. It is thus critical that enhancement criteria be developed, so that maximum progress can be made in studies of disease pathogenesis. In the past investigators have variously reported decreased time to maximal virus output, the magnitude of enhanced virus growth, fold-enhancement of infection, or the single antibody dilution at which maximal virus output occurs. Based upon a series of studies with various strains of all four dengue serotypes, and with Japanese encephalitis virus, using a variety of serums and monoclonal antibodies, we suggest the following strict criteria for establishing flavivirus antibody-dependent infection enhancement: 1) statistically significant increased production of infectious virions, as measured by quantitative assays, must be documented over most of the cycle of the cell infected in vitro, 2) serial dilution of the pre-incubated antibody source must produce an 'enhancement profile' of rising, peaking and declining production of infectious virions over at least a 3-log dilutional range, 3) enhancement titers must be related to other serologic measures of binding, 4) other causes of increased virus production must be ruled out, and 5) enhanced infection should be detected, when possible, with different virus strains and antibody sources, over a range of multiplicities of infection.

- 154 EVALUATION OF MONOCYTE-INFECTIVITY AS AN IN VITRO CORRELATE FOR
VIRULENCE OF DENGUE SEROTYPES -1, -3 AND -4.
S.C. Kliks,* L.H. Wahl and D.W. Trent. University of California at Berkeley, Berkeley, CA,
the National Institute of Dental Research, NIH, Bethesda, MD, and Division of Vector-Borne Viral
Diseases, CDC, Ft. Collins, CO.

We previously reported on a correlation between the ability of dengue-2 viral strains to infect human monocytes in the presence of enhancing antibodies and their association with severe dengue illnesses (i.e. DHF/DSS) in humans. Those studies have now been extended to include strains of dengue serotype -1, -3, and 04 viruses with varied geographic origins. Antibody-enhanced infection of human monocytes was observed for 28 of 41 (69%) strains of dengue-1 virus and 17 of 19 (89%) strains of dengue-4 virus. Monocyte-infectivity by various isolates of dengue-1 virus did not correlate with their association with DHF/DSS in humans while a positive correlation was found with isolates of dengue-4 virus ($p < 0.05$). In contrast, 19 out of 27 (70%) strains of dengue-3 failed to infect human monocytes (in the presence of enhancing antibodies), even though 70% of these isolates was associated with DHF/DSS. These findings suggest a possible divergence in pathogenetic mechanism between dengue viral serotypes-2 and -4 on one hand, and viral serotypes-1 and -3 on the other.

ASTMH POSTER SESSION I

- 155 COMPARATIVE MORPHOGENESIS OF JAPANESE ENCEPHALITIS VIRUS
*M.B. Downs, M.A. Ussery and T.P. Monath, United States
Army Medical Research Institute for Infectious Diseases,
Ft. Detrick, MD

Japanese encephalitis virus (JEV) replicates in a variety of cell types. Morphogenesis of the Beijing strain of JEV was examined in a visceraally derived cell line (VERO), a CNS derived cell line (MPF) and in neurons and glia of brains from peripherally infected mice. Viral replication in VERO cells was characterized by a proliferation of smooth membranous vesicles within the cisternae of the endoplasmic reticulum (ER). Enveloped virions were also located within the ER and in numerous cytoplasmic vesicles. Virions in MPF cells were located in cytoplasmic vacuoles of unknown derivation. Extracellular virions were adherent to the cell surface of both cell lines. Both neurons and glia from peripherally infected mice contained JEV in endoplasmic reticular cisternae, in Golgi cisternae and in coated and non-coated vesicles related to the Golgi apparatus, but not related to the plasmalemma. No extracellular virions were observed in brain tissue. Glial cells contained replicative complexes dominated by endoplasmic cisternae filled with masses of smooth vesicles similar to those seen in VERO cells. Infected neurons, however, exhibited viral complexes with markedly different ultrastructure. Dilated endoplasmic cisternae contained fewer smooth vesicles, but larger numbers of enveloped virions. The cisternae frequently radiated from electron dense structures which sometimes formed crystalline arrays. Neurons occasionally contained crystalline inclusion bodies independent of endoplasmic cisternae.

- 156 FREEZE-PRESERVATION OF NAEGLERIA FOWLERI AMEBAE. David T. John* and Penny L. Eddy. Oral Roberts University School of Medicine, Tulsa, OK.

Naegleria fowleri is a pathogenic free-living ameba and the cause of a fatal meningoencephalitis in humans. Maintenance of numerous stock cultures by frequent passage is time-consuming; hence, preservation by freezing would be desirable. Accordingly, we have defined the optimal conditions for freeze-preservation of N. fowleri amebae. Amebae were cultured in a 1:1 mix of Balamuth and Nelson ameba media supplemented with 2% calf serum and incubated at 37°C. Exponentially growing amebae were suspended in various cryopreservation preparations, placed at -20°C for 1 hr, stored at -70°C for 7 da, rapidly thawed at 37°C, and viability determined using equal volumes of 0.4% Congo red and ameba suspension. DMSO was a better cryopreservant than 3 others tested, namely, glycerol, hydroxyethyl starch, and polyvinylpyrrolidone (10,000 and 40,000 mol wts). Of 6 concentrations of DMSO tested (2, 4, 8, 10, 12, 16%), 12% produced the best viability. Heat-inactivated calf serum at 20% provided greater protection than either calf serum or heat-inactivated fetal calf serum at 10, 40, 80 or 100%. A cell concentration of 10⁶ amebae/ml yielded better viability than either 10⁵ or 10⁷ amebae/ml. Viability was improved by the addition of 10% glucose and by equilibration at room temperature for 30 min prior to freezing. Spent medium yielded greater viability than fresh medium. In summary, optimal conditions for freeze-preservation of N. fowleri amebae were: 10⁶ exponentially growing amebae/ml, 12% DMSO, 20% heat-inactivated calf serum, 10% glucose, in spent mix medium; 30 min equilibration at room temperature prior to 60 min at -20°C and storage at -70°C. Under these conditions, viability was 76% after 7 days. (Supported by Assistance Grant R-814327 from the U.S. Environmental Protection Agency.)

ASTMH POSTER SESSION I

157 A MUTANT OF Entamoeba histolytica WITH THE COLLAGENASE AND PROTEINASES ACTIVITIES CHANGED.

M. De la Garza, J. Serrano, B. Gallegos, R. Tovar and M.L. Muñoz. Centro de Investigación y de Estudios Avanzados del I.P.N. Apdo. 14-740, México, D.F. 07000, MEXICO.

In order to study the relationship among the molecular components, pathogenicity and virulence of the parasite of Entamoeba histolytica, we are characterizing variants of amoebas impaired in some functions that are important to the host-parasite relationship. BG-3, a mutant selected from the virulent strain HMI:IMSS by its resistance to cytochalasin D had the erythrophagocytosis and cytophatic effect diminished (Gallegos, De la Garza and Meza, Arch. Inv. Méd. Méx 17:87, 1986). We are now showing that BG-3 has a collagenolytic activity diminished and the proteases activity changed. We know that the parental strain secretes electron-dense granules (EDG) when trophozoites are incubated with collagen type I. Contrary, the mutant do not produce these EDG and a monoclonal (L.7.1) antibody prepared against EDG not react with BG-3. We have demonstrated that calmodulin participate in production of EDG, therefore, we study the distribution of this protein by immunoelectron microscopy. We do not find any aparent change in this clone. In conclusion, this mutant may be very helpful to study the role of EDG in the parasite pathogenicity.

Supported by grants from CONACYT (PCSACNA 050567), COSNET SEP (441.85) and Ricardo J. Zevada (83/87), MEXICO. Studies of electron microscopy were developed in the "Unidad de microscopía electrónica" del CINVESTAV, IPN.

158 BINDING OF GROWTH-INHIBITORY TRICYCLIC ANTIDEPRESSANT DRUGS TO TROPHOZOITES OF GIARDIA LAMBLIA.

E.C. Weinbach*, L. Levenbook and D. Rainey. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD

Previous studies in our laboratory have demonstrated that the antidepressant drug chlorimipramine is 3-fold more effective in suppressing growth of trophozoites of Giardia lamblia than is the parent compound imipramine. The present study is aimed at understanding the basis of this differential toxicity. Uptake of radiolabeled drugs by suspensions of trophozoites was done under various experimental conditions. Chlorimipramine uptake by G. lamblia was only 20% more than that of imipramine; this does not account therefore for the 3-fold difference in their toxicities. Uptake of the drugs was rapid; at 30° maximal accumulation occurred within 5 minutes. Both compounds, although highly lipophilic, bind to protein sites of the parasite. Delipidized trophozoites still exhibited substantial uptake of the drugs, whereas treatment of G. lamblia with protein denaturing reagents released more than 90% of the bound drugs. The drugs exhibited both non-specific and specific binding to the parasites as judged by competitive inhibition with unlabeled analogs of imipramine. Both chlorimipramine and imipramine released protein(s) from the parasites; the chloro analog being 2 to 3-fold more effective. We have found a correlation between those compounds that are weak inhibitors of parasite growth and their ability to release protein(s). These marked differences in the drugs ability to release protein may be related to the differences in their toxicities for the parasite.

ASTMH POSTER SESSION I

- 159 RECURRENT VARIATIONS OF SURFACE ANTIGENS IN CLONES OF *Giardia lamblia*.
JA. Enciso, R. Fonseca, R. Argüello, R. Cedillo and M.G. Ortega-
Pierres*. Dept. Genetics and Molecular Biology. Centro de Investiga-
ción y Estudios Avanzados IPN. México, D.F. 07360.

We have obtained *G. lamblia* isolates from asymptomatic and symptomatic mexican patients. Analysis of surface components by ^{125}I labelling showed antigenic differences among isolates. To further analyse heterogeneity within isolates, clones were obtained and analyzed by: a) immunofluorescence with MAb 3C1 which stains the surface of *G. lamblia* trophozoites and reacts by affinity chromatography with two antigens of approximately 65 and 33 Kd, b) surface labelling with ^{125}I and c) in their capacity to adhere to epithelial cells. Our results showed that percentage of fluorescence in clones from a single isolated varied. There were clones which exhibited high fluorescence (100%), others presented low values (2%) and there were some with intermediate values (40-60%). The reactivity pattern of some clones with MAb 3C1 presented recurrent variations during *in vitro* culture. Some clones remained highly reactive (100% fluorescence) for 1 to 3 months then this reactivity decreased for a short time and was regained to high levels with subsequent changes. Other clones showed low levels of fluorescence (2%) for longer periods in culture then positive staining was observed with subsequent lost and regain of reactivity. Changes in percentage of fluorescence of clones correlated with quantitative changes in the patterns of ^{125}I labelled antigens. The capacity to adhere to epithelial cells was similar in clones with either high or low percentage of fluorescence. However, when adherent assays were performed using trophozoites undergoing antigenic changes in surface components, lower number of trophozoites adhering to epithelial cells were detected. These recurrent changes in surface antigens of cloned trophozoites may be important in the pathogenicity of giardiasis.

- 160 ISOLATION OF CLONES OF *GIARDIA LAMBLIA* (WB STRAIN) WITH DISTINCT
ANTIGENIC AND ISOENZYME PROFILES. *I.A. Udezulu, G. S. Visvesvara,
D. M. Moss AND G. J. Leitch. Morehouse School of Medicine, Atlanta, Ga.
and Centers for Disease Control, Atlanta, Ga.

Antigenic heterogeneity of axenically grown *Giardia lamblia* has been demonstrated by Nash et. al (1987,88). We have isolated a number of clones of *Giardia lamblia* (WB strain) by the limiting dilution method. Two of these clones could be distinguished into two distinct phenotypes, an immunofluorescence (IF) negative (clone 1) and an IF positive (clone 2), based on their reactivity in the IF assay using the polyclonal rabbit anti-*Giardia* serum made against the parent strain. SDS PAGE and immunoblot analysis of the urea-solubilized membrane fractions of the two clones revealed that only clone 1 had a characteristic membrane-associated antigen of about 150 kda. Clone 2, however, had a low molecular weight antigen at about 29 kda that was lacking in clone 1. Moreover when the two clones were exposed to the polyclonal antiserum almost all of the organisms in clone 1 were killed whereas the majority of *Giardia* belonging to clone 2 survived. In addition to antigenic heterogeneity the two clones also differed in their banding patterns of the phosphoglucutase isoenzyme. Antibody resistant cells from both clones have been grown in culture and experiments dealing with their antigenic profile, cytotoxicity to the antibody, isoenzyme profiles, and infectivity to gerbils are underway.

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- 161 INFECTIVITY TO MONGOLIAN GERBILS AND ISOENZYME PROFILES OF FOUR STRAINS OF GIARDIA LAMBLIA ORIGINATING FROM DIVERSE GEOGRAPHIC AREAS.
*S. Abaza, G. S. Visvesvara, and J. Sullivan. Suez Canal University, Ismailia, Egypt and Centers for Disease Control, Atlanta, Ga. 30333.

Axenically cultured trophozoites of a recently isolated strain (EG) of G. lamblia from an Egyptian patient and 3 well established strains (WB, TH and VA) were inoculated per os to 4 groups (5 animals per group) of gerbils to study the infectivity and cyst excretion patterns of G. lamblia infection. All 5 (100%) gerbils inoculated with TH became infected and excreted cysts beginning on day 4 and ranging from 5×10^3 to 370×10^3 whereas 4/5 (80%) inoculated with WB strain, and 3/5 (60%) inoculated with VA strain became infected and excreted cysts beginning on day 6 and ranging from 5×10^3 to 1.2×10^6 and 5×10^3 to 1.7×10^6 respectively. However, only one gerbil (20%) inoculated with the EG strain excreted 40×10^3 cysts in its feces on one day (day 8) and was also positive for trophozoites in the intestine at necropsy. On the other hand, 80% of gerbils inoculated with WB, 60% with VA and TH were positive for trophozoites at necropsy. The 4 strains comprised 3 zymodemes, based on isoelectric focusing on polyacrylamide gels, of malic enzyme (ME), glucose phosphate isomerase (GPI), esterase (EST) and phosphoglucosmutase (PGM). The WB and VA strains were assigned to one zymodeme and EG to a second. The banding pattern of TH strain representing the third zymodeme shared common bands with both of the other zymodemes. These data indicate that distinct differences exist between strains of G. lamblia and further work is necessary to draw any correlation between these differences, transmission patterns and clinical disease.

- 162 EVALUATION OF AN ENZYME IMMUNOASSAY (EIA) FOR THE DETECTION OF GIARDIA LAMBLIA IN STOOL SPECIMENS.
L. Sloan and J. E. Rosenblatt. The Mayo Clinic and Mayo Foundation, Rochester, MN.

We evaluated a commercially produced EIA (GiardEIATM-Antibodies Incorporated) for the detection of G. lamblia antigens in 116 stool specimens which were routinely submitted to the Mayo Clinic Parasitology Laboratory. Specimens were either examined in the fresh state or were frozen at -60°C for examination at a later date. Fecal diluents were added to "test" tubes which had been coated with anti-Giardia antibody. During the rest of the procedure a second anti-Giardia antibody and enzymatic substrate are added and the development of a blue color indicates a positive reaction. EIA results were compared with those of conventional microscopic examination (CME) of direct wet preparations, concentrates, and trichrome-stained smears. 57 specimens were positive both by EIA and CME and 49 were negative by both methods; 21 of these negatives contained a total of 39 parasites (other than Giardia) belonging to 10 different species. 4 specimens were EIA positive but no Giardia were seen on CME and 6 others were EIA negative but revealed Giardia on CME (sensitivity of 91% and specificity of 93%). The EIA was equally reactive with specimens which had been examined in the fresh state and then frozen for as long as 4 months. Total EIA test time was only 30 minutes.

ASTMH POSTER SESSION I

163 USE OF DRIED BLOOD ON FILTER PAPERS AND SERUM SAMPLES FOR SERODIAGNOSIS OF HEMOTROPIC DISEASES--COMPARATIVE STUDY.

G.S.Z. Ssenyonga,* Sonia Montenegro-James, I. Kakoma, and R. Hansen.
*Makerere University, College of Veterinary Medicine, P.O. Box 7062, Kampala, Uganda and University of Illinois, College of Veterinary Medicine, Urbana, IL.

The suitability of blood collected on filter papers in comparison with corresponding fluid serum samples in the diagnosis of bovine anaplasmosis was studied using DOT-ELISA, Western Immunoblot and Rapid Card Agglutination Test (RCAT). Dried blood on Whatmans filter paper No. 1 was eluted in 1.8 ml of PBS 0.05% Tween 20 giving an initial dilution of 1:100.

The titers in both DOT-ELISA and Western Immunoblotting were similar to those obtained with the sera diluted 1:100. Filter paper samples gave lower titers in the Rapid Card Agglutination test as compared with corresponding serum samples. There was no significant difference in the titers between the eluates from filter papers stored at room temperature and those stored at 4°C. Storage even at room temperature did not significantly affect reactivity. Eluates from filter papers stored for six months at room temperature continued to give similar titers as those from freshly prepared filter papers in both DOT-ELISA and Western blot, and in the Rapid Card Agglutination test.

It is concluded that collecting blood on filter papers is a suitable technic for large scale screening and for seroepidemiological studies and offers a lot of advantages especially in developing countries where transport and cold chain facilities are a major constraint.

164 STUDIES ON THE MAINTENANCE OF *BABESIA BOVIS* (MEXICAN ISOLANT) WITHOUT SUBCULTURE USING MICROAEROPHILOUS STATIONARY PHASE (MASP) CULTURE TECHNIQUE.

Mishra, A.K.*, Clabaugh, G., Kakoma, I., and Ristic, M.
Department of Veterinary Pathobiology, College of Veterinary Medicine, Urbana, IL 61801, USA; *Present address: Division of Parasitology, Indian Veterinary Research Institute, Izatnagar 243 122, India.

A stablate of *B. bovis* was thawed and inoculated into a susceptible calf. The blood was collected when the parasitemia (PPE) reached 6% and defibrinated. The organism was maintained in MASP culture system for over 6 weeks involving 9 subcultures with maximum PPE of 13%.

Two subcultures were established for dilutions of 1:1, 1:2, 1:5 and 1:12, 1:5, 1:12 of the original PPE. No further subculture was done for the subsequent 10 days. The supernatant in each culture plate was replaced every 24 hrs with fresh culture medium containing Medium 199, 60% and normal bovine serum 40% at pH 7.0. After 96 hrs some extracellular merozoites were observed. Some erythrocytic parasite forms in poorly stained erythrocytes were also observed indicating possible metabolic changes in the host cell. On the eleventh day, two cultures in dilution 1:5 showing PPE of 8 and 5, respectively, were again separately subcultured. Seventy-two hrs following this subculture the PPE ranged from 4-10%. The morphology of the parasite was good and no extracellular forms were observed.

These data indicate that *B. bovis* can survive without subculturing in MASP culture system at 37°C, 4% carbon dioxide and 95% relative humidity for a period of at least ten days provided supernatant was regularly replaced with fresh culture medium. The survival and ability of the parasite to multiply *in vitro* was further confirmed by subsequent 8 subcultures of the parasite with maximum PPE of 11% in the same culture system under identical conditions.

ASTMH POSTER SESSION I

- 165 THE ANTIGENICITY AND IMMUNOGENICITY OF *THEILERIA SERGENTI* ISOLATED FROM KOREAN CATTLE. Baek, B.K.*, Kim, B.S., Kim, J.H., Chin, C.M., Montenegro-James, S., and Kakoma, I. College of Veterinary Medicine, Chonbuk National University, Chonju 560-756, Korea* and Department of Veterinary Pathobiology, University of Illinois, Urbana, IL 61801, U.S.A.

Theileriosis is an endemic disease in Korean cattle, manifested by anemia, hemoglobinuria, drop in milk production and loss of body weight. *Theileria sergenti* merozoites were isolated and purified from infected erythrocytes using differential centrifugation and ultrasonic disruption to prepare soluble antigen. SDS-PAGE analysis demonstrated minimal erythrocytic stroma contamination and revealed at least 15 polypeptides in the MW range of 200-14 kd. *Theileria sergenti* antigenic profile recognized by anti-*T. sergenti* antibodies demonstrated 12 polypeptide (28 kd, 30 kd, 34 kd, 36 kd, 38 kd, 41 kd, 48 kd, 56 kd, 58 kd, and 116 kd). The 28 kd, 30 kd and 41 kd were the most immunodominant. A vaccine (protein content 1.0 mg/dose) was prepared and supplemented with Freund's adjuvant or incomplete Freund's adjuvant and administered to susceptible animals. These cattle were needle challenged at the 10th week after the booster injection with a cryopreserved stabilate 1.5 ml (REC: 5.56×10^6 /dose, parasitemia: 40:1%, PCV: 50%). The antibody responses were measured by the IFA throughout the experiment. All vaccinated cattle showed protection after challenge, characterized by minimal PCV reduction, whereas control cattle suffered acute clinical disease with significant differences in all the clinopathophysiologic parameters analyzed. Western blots using sequential serum sample identified the antigens most relevant for the induction of protective immunity.

- 166 THE CLONING AND EXPRESSION OF THE CAPSULAR ANTIGEN (F1) OF *YERSINIA PESTIS* IN *ESCHERICHIA COLI* AND ITS POTENTIAL USE IN SERODIAGNOSIS AND VACCINE DEVELOPMENT. *W.J. Simpson, R.E. Thomas, T.G. Schwan and M.E. Schrupf. Arthropod-borne Diseases Section, Laboratory of Pathobiology, Rocky Mountain Laboratories, National Institutes of Health, Hamilton, MT.

Yersinia pestis is the etiological agent of bubonic plague, a zoonotic disease transmitted by fleas. At temperatures above 28°C, this Gram-negative bacillus produces a glycoprotein capsule with antiphagocytic properties. Because fleas are at ambient temperature, their bacilli lack the capsule until after several cell divisions in the mammalian host. Expression of capsular antigen induces a good immune response and vaccinating animals with a purified fraction of the capsule, fraction 1 antigen (F1), confers protection against virulent *Y. pestis*. Since F1 is specific for *Y. pestis* and animals with plague have high titers of anti-F1 antibodies, F1 forms the basis of the serological tests used in plague surveillance and diagnosis. It is presently purified from *Y. pestis* cells that are dried in acetone after culturing for 3 days at 37°C. Our objectives are to derive an improved F1 purification scheme using a highly expressed recombinant clone and evaluate the feasibility of a *Y. pestis* subunit vaccine based on F1. We now report the cloning of the F1 gene and its expression in *E. coli* using the phagemid vector λ ZAPII and a F1-specific monoclonal antibody. F1 expression was associated with a 9.4 kilobase EcoRI fragment and the recombinant protein had the same molecular mass (17,000) as F1 from *Y. pestis*. The recombinant cells produced F1 at 37°C but only minimal amounts at 28°C, indicating F1 expression is affected by temperature as it is in *Y. pestis*. Furthermore, heat-killed *E. coli* cells expressing F1 were 100% protective when immunized mice were challenged with 10^6 virulent *Y. pestis* organisms. These data indicate that F1 is readily expressed in the faster growing, yet safer bacterium, *E. coli*, and probably retains its protective immunogenic properties.

ASTMH POSTER SESSION I

- 167 INTRAGASTRIC INOCULATION OF THE PLAGUE CAPSULAR ANTIGEN CAN RESULT IN NONPROTECTIVE SERUM TITERS IN MICE. *R.E. Thomas and T.G. Schwan. Arthropod-borne Diseases Section, Laboratory of Pathobiology, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT.

Many animals, including dogs, cats, mongooses and grasshopper mice, have been shown to seroconvert to the capsular (fraction 1) antigen of *Yersinia pestis* when fed prey animals experimentally infected with plague. Fraction 1 (F1) antigen is a glycoprotein and because glycosylated proteins show resistance to proteolytic digestion, we evaluated the potential use of F1 as an oral immunogen. We hypothesized that oral immunization with the F1 antigen may be a realistic means of controlling isolated areas of epizootic plague in wildlife populations. The protective value of F1 for mice was evaluated by intragastric (IG) intubation of white mice. Mice were given IG doses of 0.1 mg, 0.2 mg, or 0.4 mg of F1 antigen at weekly intervals for three weeks. Control groups were given either 0.1 mg of F1 by intraperitoneal (IP) inoculation weekly for three weeks, or a single IG dose of F1 producing avirulent *Y. pestis* strain A1122, or IG doses of PBS. Mice were challenged with virulent *Y. pestis* in a mean lethal dose experiment three weeks after the final intubation/inoculation. While the mice given IG doses of F1 had elevated serum titers to the antigen (>1:120), only those given the lowest concentration (0.1 mg) had significantly lower mortality (3-fold protection). Those inoculated IP with 0.1 mg exhibited a 92-fold increase in protection. Results of this study suggest that oral administration of relatively large doses of F1 antigen does not lead to protective serum titers. Others have shown that oral administration of antigen can cause suppression of specific antibody production leading to immunologic tolerance. These observations should be considered if oral vaccination with F1 is to be attempted in plague control programs.

SYMPOSIUM: CYTOADHERENCE AND CEREBRAL MALARIA
(No abstracts available)

- 168 INTRODUCTION: SEQUESTRATION OF MALARIA-INFECTED ERYTHROCYTES IN VIVO. L.H. Miller. National Institutes of Health, Bethesda, MD.
- 169 PATHOLOGY OF HUMAN CEREBRAL MALARIA. M. Aikawa. Case Western Reserve University. Cleveland, OH.
- 170 IN VITRO AND EX-VIVO SEQUESTRATION ASSAYS. I.J. Udeinya. Walter Reed Army Institute of Research, Washington, DC.
- 171 INFECTED ERYTHROCYTE SURFACE MOLECULES INVOLVED IN CYTOADHERENCE. R.H. Howard. DNAX Research Institute. Palo Alto, CA.
- 172 HOST RECEPTORS FOR INFECTED ERYTHROCYTES. J. Chulay. Walter Reed Army Institute of Research, Washington, DC.
- 173 CEREBRAL MALARIA IN CHILDREN: CLINICAL IMPLICATIONS OF CYTOADHERENCE. M. Molyneux. Malaria Research Project, Blantyre, MALAWI.

D: CLINICAL TROPICAL MEDICINE

- 174 SKELETAL AND CARDIAC MUSCLE INVOLVEMENT IN SEVERE, LATE LEPTOSPIROSIS.
*G. Watt, L.P. Padre and M.L. Tuazon. Naval Medical Research Unit
No. 2, Manila, Philippines.

Data is lacking on the extent and prevalence of heart and skeletal muscle injury in late leptospirosis. We therefore studied 38 hospitalized patients in the Philippines with proven severe leptospirosis (Weil's disease) for evidence of both myocardial and striated muscle involvement and indications of a relationship between the two types of muscle damage. Daily physical examinations focused on the cardiovascular system and detection of myositis. Serum was assayed for creatinine phosphokinase activity and standard 12 lead electrocardiograms (EKGs) were obtained on admission, during hospitalization and at follow-up. Patients presented late in the course of leptospirosis. Thirty-seven percent of the patients had myositis and 48% had clearly abnormal EKG findings. First-degree atrioventricular heart block and changes suggestive of acute pericarditis were the two most common EKG abnormalities. Two patients had pericardial friction rubs but none had pericardial effusion, shock or congestive heart failure. There was no association between damage to skeletal muscle and damage to heart muscle ($p = 0.35$), though both were associated with particularly severe disease. ECG changes and myositis therefore occurred commonly in patients with Weil's disease but clinically significant cardiac manifestations were unusual. Skeletal and cardiac muscle involvement were associated with especially severe disease but not with each other, suggesting different pathogenic mechanisms.

LATE STAGE EAST AFRICAN HUMAN TRYPANOSOMIASIS - TREATMENT AND FOLLOW-UP

- 175 *J.D. Bales, S.M. Harrison, D.L. Mbwabi, K.M. Tengekyon, A. R. Njogu, P.J. Schechter. Fitzsimons Army Medical Center, Aurora CO; Kenya Trypanosomiasis Research Institute, Nairobi, Kenya; Merrell Dow Research Institute, Strasbourg, France.

We evaluated a World Health Organization treatment protocol for late stage East African Human Trypanosomiasis in forty Kenyan cases followed for three years. We observed five deaths (12.5% death rate) and three relapses (7.5% relapse rate). All deaths occurred either before, during, or within three months of initial therapy. Relapses occurred at 3, 12, and 19 months post treatment. The relapse cases were treated with Difluoromethyl Ornithine (DFMO, Eflornithine). Two of the three treated with DFMO relapsed at 5 and 12 months following therapy. We conclude that late stage East African Human Trypanosomiasis remains a disease with significant early mortality and potential for relapse despite accepted therapy. We also conclude that DFMO monotherapy is unsuccessful in treating arsenical refractory late stage East African Trypanosomiasis.

D: CLINICAL TROPICAL MEDICINE

- 176 KATAYAMA FEVER IN U.S. TRAVELERS RETURNING FROM BOTSWANA. POSSIBLE ROLE OF A HYBRID SCHISTOSOME SPECIES. M. K. Michelson*, J. J. Sullivan, L. Chitsulo, M. Wilson, H. S. Bishop, D. D. Juranek, and H. C. Spencer. Parasitic Diseases Branch, CDC, Atlanta, GA.

In December 1988, 11 (69%) of 16 travelers returning from an expedition to the Okavango Delta region of Botswana experienced a constellation of symptoms which included headache (91%), tiredness (91%), fever (55%), sweating (55%), chills (45%), weight loss (45%), myalgias (36%), anorexia (36%), diarrhea (18%), and cough (18%). Peripheral eosinophilia was detected in 6 (38%) individuals and ranged from 10% to 57% of circulating granulocytes. 15 of 15 serum specimens tested were positive for antibodies to Schistosoma species on screening FAST-ELISA. However, none of these 15 specimens were reactive for S. mansoni or S. haematobium on initial immunoblot analysis. 9 (82%) of 11 stool specimens submitted to CDC were positive for small numbers of eggs resembling those produced by a Schistosoma mansoni/Schistosoma rodhaini hybrid. All individuals were advised to take a single oral dose of praziquantel 40 mg/kg body weight. No subsequent relapses have been reported following therapy. This cluster of cases illustrates the potential importance of variant Schistosome strains in causing human disease and the difficulties associated with their detection on routine stool examination.

- 177 MALARIA IN ADULTS AT A MAJOR MEDICAL CENTER IN NEW YORK CITY 1971-1987. *J. M. Courval & S. H. Vermund. Columbia University, Epidemiology, NYC; NIAID, Bethesda.

The Centers for Disease Control have expressed concern about whether malaria is being diagnosed and treated appropriately in the United States, particularly because a few deaths due to malaria occur each year. We describe the population characteristics and evaluate the diagnosis and treatment of adult malaria patients seen at a major medical center in New York City from 1971 to 1987.

Methods: Cases were identified by computerized hospital discharge diagnosis and from a log kept by the parasitology laboratory. Information was collected by medical chart review of blood smear confirmed cases.

Results: We identified 50 episodes of malaria in 47 patients with no fatalities. One case was acquired by blood transfusion. The parasitology laboratory log identified 45 cases; discharge diagnosis search, 23. Malaria was suspected in most patients and a malaria smear ordered, but in 3 cases diagnosis was made from the WBC differential smear. Malaria species distribution was 45 % vivax, 23 % falciparum, 17 % malariae, remainder ovale, mixed, undetermined.

Almost all falciparum malaria cases were hospitalized, median length of stay 5.0 days; fewer than 50 % of other cases were hospitalized, median length of stay 4.5 days. Most malaria infections were diagnosed within 24 hours of a patient's first visit, but 1 falciparum case was not diagnosed until day 4. Treatment, in general, followed current recommendations and was successful. An exception was a falciparum patient in 1984 with multiple African travel. This patient still had fever and headache almost 48 hours after start of chloroquine. Therapy for chloroquine resistant falciparum was begun at the patient's insistence and the symptoms resolved.

Africa was the source of infection for 15 out of the 19 cases in U.S. citizens; in foreign nationals about 40 % were acquired in Africa and 23 % in Asia. More than 50% of U.S. citizens, but fewer than 20 % of foreign nationals appeared to have been asked about use of prophylaxis. Recommendations for prophylaxis or other methods of control in case of future travel were noted in fewer than 20 % of all cases.

Conclusions: The epidemiology of malaria at this medical center mirrored the overall picture in the U.S. during this period. Diagnosis was not always prompt, but treatment of malaria was in general appropriate. History taking of prophylaxis information and recommendations for future travel could be improved. Computerized discharge diagnosis identified only 50 % of the total cases.

D: CLINICAL TROPICAL MEDICINE

INTRAMUSCULAR QUININE IN THE TREATMENT OF SEVERE PEDIATRIC FALCIPARUM 178 MALARIA.

Terrie E. Taylor*, Malcolm E. Molyneux, Jack J. Wirima. Michigan State University, Liverpool School of Trop Medicine, Kamuzu Central Hospital.

Quinine remains the drug of choice in the treatment of severe chloroquine-resistant falciparum malaria. Intravenous infusions are safe and effective, but are frequently not feasible in rural settings where the majority of patients are treated. Few studies of intramuscular quinine (IM Qn) have been done and there is no consensus regarding the efficacy and safety of giving quinine by this route.

We have compared the pharmacokinetics, the effect on blood glucose and plasma insulin concentrations, and the efficacy of three different regimens of IM Qn in Malawian children who required parenteral treatment for falciparum malaria because of intractable vomiting or altered consciousness.

Three dosage schedules were evaluated:

A - 10 mg/kg eight-hourly (n=9)

B - 20 mg/kg initially, followed by 10 mg/kg eight-hourly (n=10)

C - 10 mg/kg four-hourly x 2, then eight hourly (n=10)

All three were equally efficacious in terms of parasite- and fever-clearance times. IM Qn is rapidly absorbed, and the effect on insulin secretion is proportional to the rate of rise in plasma Qn concentration. In this study, patients on regimen C achieved maximum plasma concentrations of Qn rapidly, but none developed hyperinsulinemic hypoglycemia.

Based on our findings, regimen C appears to be a safe and effective method of administering IM Qn, and could greatly simplify the management of severe falciparum malaria in settings where intravenous treatment is not possible.

GLUCOSE METABOLISM IN QUININE-TREATED FALCIPARUM MALARIA 179

*T.M.E. Davis, W. Supanaranond, S. Pukrittayakamee, S. Krishna, S. Looareesuwat, R.C. Turner and N.J. White. Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Nuffield Department of Clinical Medicine and Diabetes Research Laboratories, Oxford University, Oxford, England.

Hypoglycemia in falciparum malaria results both from the disease and quinine therapy. To investigate host and drug effects on glucose metabolism, ten non-hypoglycemic, normal-weight, untreated, fasting Thai males with parasitemia $< 100,000/\mu\text{l}$ were studied. Each underwent a two-hour glucose infusion (5 mg/kg ideal body weight (BW).minute) with an infusion of quinine dihydrochloride (10 mg/kg BW) during the second hour. Eight patients returned for restudy during convalescence. Mean fasting plasma glucose and insulin concentrations were higher during acute illness ($p < 0.001$ and $p = 0.058$ respectively). Mathematical model assessment of one-hour glucose and insulin concentrations ('CIGMA') revealed lower tissue insulin sensitivity on admission (geometric mean $[-1\text{SD} - +1\text{SD}]$; 97% [71-134]) than in convalescence (139% [109-178], $p < 0.025$). Two-hour plasma glucose (9.5 ± 2.0 mmol/l) and insulin (81.8 mU/l [51.5-129.9]) concentrations during acute illness were significantly higher than those in convalescence (7.2 ± 1.2 mmol/l and 40.1 mU/l [23.5-68.4], $p \leq 0.025$) despite similar simultaneous plasma quinine concentrations ($p > 0.1$). These data suggest insulin resistance in acute infection but augmented quinine-stimulated insulin secretion. Hypoglycemia occurring after successful initial treatment might thus result from increasing insulin sensitivity in the presence of continuing hyperinsulinemia.

D: CLINICAL TROPICAL MEDICINE

- 180 MALARIA CHEMOPROPHYLAXIS WITH PROGUANIL/SULFAMETHOXAZOLE.
JJ Karwacki, GD Shanks*, V Suriyamongkok, C Watanasook.
Armed Forces Research Institute of Medical Sciences,
Bangkok, Thailand and Royal Thai Navy Medical Department.

The difficulty of providing effective malaria chemoprophylaxis in South East Asia is increasing. New combinations of antifolates/para-aminobenzoic acid inhibitors may be useful despite wide-spread pyrimethamine/sulfadoxine resistance. Thai soldiers (N=360) on the Thai-Kampuchean border were entered into a randomized malaria chemoprophylactic trial. Proguanil (200 mg/day) combined with sulfamethoxazole (1000 or 1500 mg/day) were compared to a standard combination of weekly pyrimethamine/dapsone (Maloprim). Men receiving proguanil/sulfamethoxazole had a significantly lower malaria attack rate than those taking pyrimethamine/dapsone. This was true both in the first five week phase utilizing 1000 mg of sulfamethoxazole (.11 vs .26) and in the second ten weeks utilizing 1500 mg of sulfamethoxazole (.13 vs .30). P. vivax was prevented significantly better by proguanil/sulfamethoxazole ($p < .001$) with a relative efficacy of 70% during both phases. P. falciparum was prevented significantly better by proguanil/sulfamethoxazole ($p < .001$) with a relative efficacy of 43% when both phases were combined. Unenforced compliance was 90% in both groups receiving daily medicine. No serious drug side-effects were observed. Proguanil/sulfamethoxazole may represent a useful chemoprophylactic option in areas of multiple drug resistant malaria.

- 181 PERMETHRIN-IMPREGNATED CURTAINS AND BED NETS PREVENT MALARIA IN WESTERN KENYA. *J.D. Sexton, T.K. Ruebush II, A.D. Brandling-Bennett, J.G. Breman, S.J. Odera, J.M. Roberts and J.B.O. Were. Division of Parasitic Diseases, CDC, Atlanta, GA, USA; Kenya Medical Research Institute, Nairobi, Kenya.

In 1988, a 15-week study was conducted in Uriri, Kenya, to evaluate the effectiveness of permethrin-impregnated (0.5 g/m^2) bed nets and curtains as a malaria control measure. 105 families were randomly assigned to control, bed net, and curtain study groups. Baseline malaria prevalence was 75%; all participants were cured of parasitemia with pyrimethamine/sulfadoxine. Blood smears were taken weekly and clinical interviews were conducted twice a week. Anopheles mosquitoes resting indoors were collected weekly. Malaria knowledge surveys were conducted at the beginning and end of the study. During the 1384, 1463, and 1447 person-weeks that the controls, bed net users, and curtain users were at risk, respectively, there were 75, 55, and 34 newly acquired Plasmodium falciparum infections, respectively. Infections per person-weeks at risk were significantly higher for the control group than for either the curtain users ($p < 0.001$) or bed net users ($p = 0.04$). 45% of the bed net and curtain users and 30% of those in the control group reported no episodes of fever and chills. This difference was significant. Resting Anopheles gambiae or An. funestus were found on 100 occasions in the control houses but only twice in the other houses. Indoor pyrethrum knockdown collections produced 195, 23, and 3 An. gambiae and An. funestus from the control, bed net, and curtain houses, respectively. Permethrin-impregnated nets and curtains decreased incidence of malaria infections and number of anopheline vectors, and curtains appeared to be more effective than nets in this study. Supported by AID/VBC PASA DTE-5948-P-H-C-6052.

E: ARBOVIRAL ENTOMOLOGY

- 182 VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION IN AND TRANSMISSION BY AMBLIOMMA CAJENNENSE (FABRICIUS). K.J. Linthicum,* T.M. Logan, C.L. Bailey, S.W. Gordon, C.J. Peters, T.P. Monath, J. Osario, D.B. Franc, R.G. McLean, J. LeDuc, R.R. Graham, J. Moulton, and D. Dohm. U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD and Centers for Disease Control, Ft. Collins, CO.

The mechanism for interepizootic maintenance of epizootic variants (I-ABC) of Venezuelan equine encephalomyelitis (VEE) virus remains an enigma. Investigations which have examined the potential for a vector role in the maintenance of the virus have focused almost exclusively on looking for a mosquito-vertebrate cycle. A possible tick-vertebrate maintenance cycle has been conspicuously overlooked. The larvae, nymphs, and adults of Amblyomma cajennense, the most commonly encountered and aggressive Central and South American tick, are nonspecific feeders which occur throughout the geographic range of epizootic VEE. To assess the potential for A. cajennense to serve as a vector/maintenance host for VEE virus, larval and nymphal ticks were allowed to feed on strain-13 guinea pigs that had been inoculated with an epizootic strain (Trinidad donkey) of VEE virus. Virus replicated in the larvae following ingestion of ca. $10^{3.8}$ plaque-forming units (PFU) and was transstadially transmitted to the nymphs, persisting at least 88 days in unfed specimens (mean titer = $10^{2.7}$ PFU). Nymphs, infected as larvae, transmitted the virus to guinea pigs while feeding. Virus was transmitted transstadially to adults, but these adults (0/80) did not transmit the virus during feeding. Virus was not recovered from any of 251 nymphs which had feed on viremic guinea pigs 2-43 days previously. These data suggest that epizootic VEE virus strains could be maintained and transmitted by A. cajennense ticks infected as larvae in a possible tick-rodent cycle.

- 183 DETECTION OF ARBOVIRUS DEPOSITION IN MOSQUITOES FOLLOWING INGESTION OF RADIOLABELED VIRUS IN BLOOD MEALS. *S. C. Weaver, T. W. Scott, L. H. Lorenz and P. M. Repik. Dept. of Entomology, University of Maryland, College Park, Maryland 20742 and Dept. of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19130.

We examined deposition of eastern equine encephalomyelitis virus (EEEV) in the alimentary tract of its mosquito vector, Culiseta melanura, to detect potential sites of initial infection. Artificial viremias were created by injecting purified ^3H - or ^{35}S -labeled EEEV intravenously into one day old chicks. Mosquitoes were allowed to engorge, incubated 1-2 hr, fixed, and whole tagmata of 20 specimens embedded. Serial 1 μm sagittal or transverse sections were cut through all specimens, mounted on slides and coated with autoradiographic emulsion. Following exposures of 1-2 months, slides were developed and examined by phase contrast or Nomarsky microscopy.

All 20 mosquitoes contained virus in the posterior midgut lumen, as well as within luminal folds at the cardiac midgut-intussuscepted foregut junction. Virus was not detected within the diverticula or foregut. Posterior midgut virus was concentrated in a band of expressed plasma adjacent to the epithelium. In addition, 2/20 specimens contained virus in the abdominal hemocoel and/or fat body. One mosquito contained chick blood cells in the hemocoel, suggesting a leaky midgut. Blood cells and virus were also detected within the pyloric ampulla of 3/20 mosquitoes.

These findings are consistent with traditional views of initial posterior midgut infection. They also suggest the possibility of early anterior midgut and/or foregut infection. However, other studies have yielded no evidence of early EEEV infection at this site. Leaky posterior midguts exhibited by some mosquitoes may be important in facilitating rapid systemic infections.

NUMBER OF EGGS PER SITE LAID BY Aedes aegypti AND IMPLICATIONS FOR DISPERSAL AND VIRUS TRANSMISSION. P. Reiter,* M.A. Amador and D.J. Gubler. Dengue Branch, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, GPO Box 4532, San Juan, Puerto Rico 00936.

It is generally accepted that an Aedes aegypti female does not deposit all her eggs at one oviposition site. We examined the frequency of the numbers of eggs laid in 1000 ovitraps in San Juan, Puerto Rico, and found clear peaks at a mean interval of about 23 eggs. We believe that these peaks represent multiples of the average number of eggs laid by individual females per site-visit. From the number of eggs in fully gravid, wild caught mosquitoes, we deduce that a female Ae. aegypti must find up to eight suitable sites to deposit her entire batch of eggs. If we assume that mosquitoes do not lay more than once at any particular site, we can predict that an individual female must search at least 15 of the domestic properties in our study area during every oviposition cycle. The time required for searching and selecting acceptable oviposition sites is not known, but is likely to span more than one circadian cycle, especially in large insects. During daily periods of inactivity, many mosquitoes rest indoors, in close proximity to man. In collections from indoor resting sites, we found Ae. aegypti with fully developed eggs and a recent blood meal. Insects in this condition have been described on several occasions in the literature, and sometimes comprise more than 50% of landing collections. We suggest that in a container-breeding vector, such as Ae. aegypti, feeding by gravid females that have laid some of their eggs is influenced by the availability of oviposition sites, and could be a significant factor in the dynamics of arbovirus transmission.

185 TRANSFILIAL TRANSMISSION STUDIES OF ST. LOUIS ENCEPHALITIS VIRUS IN
AEDES TAENIORYNCHUS AND PSOROPHORA TOLTECUM.

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Two species of mosquitoes, Psorophora toltecum (formerly Ps. columbiae) and Aedes taeniorynchus, were examined for vertical transmission of St. Louis encephalitis (SLE) virus in an effort to elucidate one possible mechanism whereby SLE virus might be maintained in nature through the winter months in southern California. Both species have distributional patterns coincident with the isolation of SLE virus in nature, overwinter in the egg stage, and hatch throughout the summer and fall with adult populations reaching their highest levels in late summer. Viral strains of SLE isolated in California from Culex mosquitoes were used to infect parental females collected from the field (Ps. toltecum) or from colonies (Ae. taeniorynchus). Females were inoculated intrathoracically with virus and held through several ovarian cycles. The F-1 progeny were subjected to various temperature regimes during larval development and pupation in order to determine whether such factors are important to the maintenance and expression of SLE virus in mosquito tissues. Several assay systems were used to detect both infectious and non-infectious virus in larval and adult progeny including a standard plaque assay, an in situ enzyme immunoassay and a cDNA probe. Results indicate that SLE virus is transmitted vertically in Ps. toltecum when pupation occurs at 21°C, although at a very low level. However, SLE virus was not transmitted vertically in three populations of colonized Ae. taeniorynchus collected from the coast of California. Larvae and adults of both species were collected from the field and assayed for the presence of SLE virus with negative results. These studies would indicate that neither species is required for the maintenance of SLE virus in nature.

E: ARBOVIRAL ENTOMOLOGY

- 186 TRANSMISSION OF RIFT VALLEY FEVER VIRUS BY THE SAND FLY, PHLEBOTOMUS DUBOSQI. M.J. Turell* and P.V. Perkins.
Department of Arboviral Entomology, USAMRIID, Fort Detrick, Frederick, MD and Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C.

Studies were conducted to determine if the sand fly, Phlebotomus dubosqi, could serve as a vector of Rift Valley fever (RVF) virus. All sand flies became infected after intrathoracic inoculation, and all (31/31) feeding, inoculated flies transmitted RVF virus to hamsters. After feeding on a hamster with a RVF viremia (ca. 10^9 PFU/ml of blood), 50% (72/145) became infected, and 80% (4/5) of sand flies with a disseminated infection (i.e., virus recovered from their legs) transmitted virus by bite. This is the first demonstration of oral transmission from animal to animal of RVF virus by a phlebotomine sand fly. None of 331 progeny of inoculated sand flies or 230 progeny of orally exposed sand flies contained virus. These studies demonstrate the potential for sand flies to serve as a vector of RVF virus in Africa and become involved in the natural transmission cycle of this virus.

- 187 CRIMEAN-CONGO HEMORRHAGIC FEVER IN SENEGAL: TEMPORAL AND SPATIAL PATTERNS OF INFECTION RELATED TO VECTOR BIOLOGY.
Mark L. Wilson*, Bernard LeGuanno, Jean-Paul Gonzalez, Jean-Paul Cornet, and Jean-Louis Camicas. Institut Pasteur, Dakar, Senegal; USAMRIID, Ft. Detrick, Frederick, MD 21701; Laboratoire ORSTOM de Zoologie medicale, c/o Institut Pasteur, Dakar, Senegal.

Transmission of Crimean-Congo Hemorrhagic Fever virus appears to be enzootic in Senegal, however the ecological factors that influence the temporal and spatial dynamics remain obscure. Our studies have shown that the geographic distribution of infection is correlated with the distribution of certain vector tick species. Studies of tick abundance have demonstrated large seasonal and annual variation. Prospective sampling of individually-identified domestic animals has exposed an epizootic that occurred during a period when adult *Hyalomma* ticks were unusually abundant. Different forms of frequency distributions of antibodies correlated with other indicators of virus circulation. Maternal transmission, detected in most offspring of seropositive sheep, may destabilize, temporally, horizontal transmission. Additionally, we studied the development and duration of antibody titers among experimentally and naturally infected animals. IgM titers developed within days after infection and persisted for weeks or months. IgG titers rose similarly, but remained detectable for up to 5 years. Other studies of the feeding pattern of *H. truncatum* demonstrated host specific variation in the length of feeding and diurnal pattern of detachment. Certain effects on the maintenance of transmission are discussed.

REPLICATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS
(FAMILY BUNYAVIRIDAE, GENUS NAIROVIRUS) IN FOUR SPECIES OF IXODID TICKS
(ACARI: IXODIDAE) AFTER EXPERIMENTAL INFECTION.

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Three, Cairo, Egypt.

The vector potential of Hyalomma dromedarii, H. impeltatum, H. truncatum, and Phipiccephalus appendiculatus for Crimean-Congo hemorrhagic fever (CCHF) virus (IbAr 10200) was evaluated by intracoelemic inoculation. All 3 Hyalomma species became infected with infection rates ranging between 80-100% at 7 to 14 days postinoculation, and viral titers increased in unfed specimens almost 100-fold above inoculation levels within the first week following infection. Only 40% of the R. appendiculatus became infected and viral titers in unfed specimens increased less than 10-fold above inoculation levels. The virus persisted up to 153 days in unfed H. impeltatum. Viral titers were significantly higher in female vs male H. dromedarii, H. impeltatum, H. truncatum, and R. appendiculatus after blood feeding. Blood feeding had little effect on the viral titers of male Hyalomma species. However, the percentage of both female and male ticks from which virus was recovered was significantly higher from fed ticks as compared to unfed ticks. No virological evidence of transovarial transmission was found in more than 78,000 first-generation progeny (larvae, nymphs, and adults) of inoculated female H. dromedarii, H. impeltatum, H. truncatum, and R. appendiculatus. All species transmitted CCHF virus to guinea pigs when allowed to feed at both 6 and 21 days postinoculation.

189 ISOFEMALE LINE ANALYSES OF THE GENETIC BASIS OF ORAL
SUSCEPTIBILITY OF CULICOIDES VARIIPENNIS FOR BLUETONGUE
VIRUS.

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Agriculture, Agricultural Research Service, Arthropod-borne
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Isofemale lines of Culicoides variipennis are being studied using an antibody capture ELISA technique to determine variation in oral susceptibility to bluetongue virus. Families orally infected with bluetongue virus have been screened using ELISA for several generations. Significant differences were detected among families. Several families have been identified which show consistently high infection rates (>70 %) while other families have shown consistently low infection rates (0-10 %). The ELISA method has allowed a quantitative analysis of oral infection in the species. The family means of individual ELISA values were significantly different and several families show low variances for ELISA values. The isofemale line analysis using a quantitative ELISA assay for bluetongue virus allows identification and isolation of phenotypes which will be useful for the analysis of the genetic and environmental components of oral susceptibility to infection. The significance of the findings for the genetic analysis of insect vector competence will be discussed.

F: SCHISTOSOMIASIS - IMMUNOPATHOGENESIS
AND MECHANISMS OF IMMUNITY

190 CHARACTERIZATION OF TH1 VERSUS TH2 CELL FUNCTION IN MICE VACCINATED
WITH RADIATION-ATTENUATED CERCARIAE.

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We have previously shown that the induction of cell-mediated immune responses including production of gamma interferon (IFN) and activation of macrophages for larval cytotoxicity correlate with resistance to challenge *Schistosoma mansoni* infection in mice vaccinated once with irradiated cercariae whereas others have implicated humoral resistance mechanisms by demonstrating that sera from multiply vaccinated mice can transfer protection. In an attempt to resolve this controversy, we have compared the reactivity of different subsets of helper T lymphocytes (Th1 and Th2) in C57BL/6 mice vaccinated either once (1x) or three times (3x) with 500 50 Kr irradiated cercariae. Levels of IFN produced by spleen or lymph node lymphocytes from 1x-vaccinated mice upon in vitro stimulation with schistosome antigens were approximately two-fold higher than those produced by cells from 3x-vaccinated mice under equivalent conditions. In contrast, IL-5 production was higher by cells from 3x-vaccinated animals. Likewise, sera from 1x-vaccinated mice contained higher levels of IgG2a antibodies against soluble schistosome antigens, while sera from 3x-vaccinated mice contained higher levels of IgG1 antibodies. These observations are consistent with the hypothesis that a single vaccination with irradiated cercariae preferentially stimulates the Th1 subset of lymphocytes involved in IFN production and macrophage activation, whereas multiple immunization increases Th2 activity involved in eosinophilia and B cell help for IgG1 and IgE responses.

191 SELECTIVE PRODUCTION OF CYTOKINES ASSOCIATED WITH THE
TH2 RESPONSE BY SPLENCYTES, DRAINING LYMPH NODE CELLS
AND ISOLATED GRANULOMAS DURING ACUTE MURINE INFECTION

WITH *SCHISTOSOMA MANSONI*. J.M. Grzych*, E. J. Pearce, A.W. Cheever, P. Scott
and A. Sher. Immunology and Cell Biology and Host-Parasite Relations Sections,
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In order to identify the subset of T helper cell involved in pathology, lymphocytes from C3H/HEN mice acutely (i.e., 8wk) infected with *S. mansoni* were stimulated with either soluble schistosome egg antigen (SEA) or concanavalin A (CON-A) and supernatants from the cultures assayed for specific cytokines by either ELISA or bioassay. Splenocytes or mesenteric lymph node cells from the infected mice produced large quantities of the TH2 specific cytokine, IL-5, with respect to the levels produced by cells from uninfected mice, after exposure to either SEA or CON-A. In contrast, the same supernatants contained marginal or undetectable amounts of the TH-1 specific cytokine, IFN- γ . Lymphocytes from mice inoculated with eggs displayed the same preferential IL-5 response. Granulomas were isolated from liver tissue of the 8 wk infected animals and cultured alone or in the presence of SEA or CON-A. Significant quantities of IL-5 and IL-4 but not IFN- γ or IL-2 were detected in the supernatants. These TH-2 cytokines were constitutively produced by the granulomas without the addition of antigen or mitogen. The above findings suggest that T cells belonging to the TH2 subset predominate in the acute granulomatous response and therefore are likely to play a role in the induction of egg pathology. This hypothesis contrasts with the established view of the schistosome granuloma as a "delayed hypersensitivity reaction" since the latter immune response is usually mediated by TH1 lymphocytes.

F: SCHISTOSOMIASIS - IMMUNOPATHOGENESIS
AND MECHANISMS OF IMMUNITY

192 IL-2 PRODUCTION, RESPONSIVENESS, UTILIZATION AND RECEPTOR DISPLAY
BY SPLEEN CELLS OF S. MANSONI INFECTED MICE.

T. Yamashita and* D.L. Boros, Wayne State U. Sch. of Med., Detroit, MI.
Peak interleukin-2 (IL-2) production in spleens of S. mansoni infected mice coincided with maximal liver granuloma responses at 8 w. and declined with the downmodulation of the response by 20w. of the infection. Restoration of modulated granuloma response by injected recombinant (r) IL-2 indicated a key role for IL-2 in granuloma formation. Therefore, we examined IL-2 related reactivity of T cell subsets in splenic cells (SPC) of infected mice. IL-2 production assayed by CTLL cell proliferation units/ml \pm SD/5x10⁶ SPC: unseparated SPC 8w: 34.8 \pm 16, L3T4⁺T_H (helper) cells: 36.4 \pm 10, Lyt2⁺T_S (suppressor) cells: 15.1 \pm 8. Unseparated 20w. SPC: 1.3 \pm 1, T_H cells: 2.3 \pm 1, T_S cells: 1.9 \pm 1. Utilization (%) of 20 u rIL-2 added to 5x10⁶/ml SPC for 48 h culture: Unseparated SPC 8w: 65.3 \pm 9, 20w: 57.9 \pm 9 (p<.01). T_H 8w: 60.2 \pm 3, 20w: 53.8 \pm 6 (p<.01), T_S 8w: 63.7 \pm 4, 20w: 52.8 \pm 11 (p<.01). Addition of 20 u of rIL-2 to 20w SPC cultures with or without 10 ug soluble egg antigens (SEA) did not restore low IL-2 production: SPC+SEA: 4.4 \pm 4, SPC+rIL-2: 6.3 \pm 5, SPC+SEA+rIL-2: 5.1 \pm 4. The percent \pm SD of IL-2 receptor (R) positive SPC was analyzed by 2 color flow cytometry using fluorescein and phycoerythrin coupled monoclonal antibodies. Results show diminished numbers of receptor-bearing T_H cells among 20w SPC. T_H 8w cells: 4.8 \pm 2, 20w cells 2.2 \pm 0.5; T_S 8w cells: 0.8 \pm 0.6, 20w cells: 0.6 \pm 0.2. It is concluded, that SPC of 20w infected mice contain fewer IL-2R⁺T_H cells, produce and utilize less IL-2 than SPC of 8w infected animals. The nature of this impairment remains to be elucidated. Impaired IL-2 production, utilization diminish inflammatory T_H cell activity and thus contribute to the down-modulation of the granulomatous response. Supported by NIH Grant AI-12913.

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CHARACTERIZATION OF A HUMAN SERUM FACTOR THAT INHIBITS CYTOKINE
MEDIATED ACTIVATION OF HUMAN EOSINOPHILS AND NEUTROPHILS. M.S.
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and Brigham and Women's Hospital, Boston, MA 02115.

Eosinophil Cytotoxic Inhibitor (ECI), which suppresses killing of S. mansoni larvae by monokine activated eosinophils, was purified from human serum. It was observed that purification methods involving size exclusion or anion exchange HPLC increased the inhibitory titer of ECI by a factor of 50-2000. This effect was dependent on exposure to silica particles and did not occur with exposure to, for example, polyacrylamide beads. At high concentrations, purified ECI appeared to have auto-proteolytic activity. However, the biological activity of ECI did not depend on proteolysis. ECI did not degrade 125 I-labeled test polypeptides substrate, nor did treatment of activated ECI with diisopropyl fluorophosphate (DFP) affect its inhibitory titer. In addition to its effect on eosinophils, ECI inhibited neutrophil adherence to plastic surfaces as well as neutrophil release of H₂O₂ following stimulus with 0.25 uM calcium ionophore A23187 (50% decrease), 2.5 uM opsonized zymosan (25% decrease), 1nM f-met-leu-phe (50-90% decrease), and suboptimal doses (5pg/ml) of phorbol myristate acetate (PMA, 30% decrease). ECI did not affect eosinophil or neutrophil exclusion of trypan blue or H₂O₂ release following optimal PMA. Thus, ECI is a potential inhibitor of cytokine mediated inflammatory responses involving eosinophils and neutrophils.

F: SCHISTOSOMIASIS - IMMUNOPATHOGENESIS
AND MECHANISMS OF IMMUNITY

- 194 HUMAN EOSINOPHIL CYTOTOXICITY-ENHANCING FACTOR (ECEF): PURIFICATION,
N-TERMINAL AMINO ACID SEQUENCE, IDENTIFICATION OF SECRETED AND
CELL-ASSOCIATED FORMS

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Human monocytes or U937 cells release a substance (ECEF) that enhances eosinophil killing of *Schistosoma mansoni* larvae. In order to purify this substance, medium conditioned by PMA/LPS-stimulated U937 cells was processed by a sequence of phenyl-Sepharose chromatography, DEAE-cartridge chromatography, preparative SDS gel electrophoresis, and reversed-phase HPLC. This resulted in the isolation of a 10 kDa polypeptide that enhanced eosinophil killing of schistosomula targets by a mean of 206% in 21 experiments (increase from $13.2 \pm 7.9\%$ of targets killed to $40.4 \pm 20.2\%$, $p < 0.0001$). The activity of ECEF was maximal at 20 ng/ml and half-maximal between 0.8 and 4 ng/ml. The following N-terminal amino acid sequence was determined for purified ECEF:

VKQIESKTAFQKAL??AG?KL. Computer search showed that this sequence is unrelated to other known protein sequences. The Ig fraction of serum from rabbits immunized with purified ECEF reacted with the polypeptide in Western blot analysis and, when precipitated by protein A-Sepharose beads, removed ECEF activity from solution. Immunoprecipitation studies with ^{35}S -methionine/cysteine-labeled U937 cells identified a 13 kDa secreted form of ECEF and a 10 kDa form that is predominantly cell-associated. Thus we have identified the physical characteristics and part of the primary structure of a monokine that is a potential regulator of eosinophil function in inflammation. The biological significance and the structural relationship of the secreted and cell-associated ECEF forms are not known at present.

- 195 SCHISTOSOMA MANSONI: EFFECTS OF PRAZIQUANTEL ON CAPPING AND CALCIUM
UPTAKE IN ADULT WORMS.

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Adult male *S. mansoni* incubated in host antigen specific colloidal gold labeled antibodies were observed to translocate those antibodies to the apical surfaces of the dorsal tubercles. This capping process occurred within 8 minutes at 37°C, was accompanied by a significant uptake of Ca^{45} by the parasite, and culminated with the expulsion of the antibody from the worm's surface. In the presence of Praziquantel capping was more rapidly accomplished (within 2 minutes) and calcium uptake was 3X higher than in the presence of antibody alone, but the parasite was unable to shed the antibody probe. (Supported by NIH Grant AI-21839)

F: SCHISTOSOMIASIS - IMMUNOPATHOGENESIS AND MECHANISMS OF IMMUNITY

- 196 PURIFICATION OF SCHISTOSOMAL EGG-GRANULOMA DERIVED FIBROBLAST GROWTH FACTOR. * Sadhana Prakash and David J. Wyler, Div. of Geographic Medicine and Infectious Disease. New England Medical Center Hospitals and Tufts University School of Medicine, Boston, MA.

Fibroblast hyperplasia is a characteristic pathological feature of hepatic fibrosis due to schistosomiasis. We have identified the presence of a growth factor for fibroblasts in culture supernatants of isolated granulomas (granuloma supernatant, GS) from liver of *S. mansoni* - infected mice. Our observations suggest a molecular link between granulomatous inflammation and subsequent hepatic fibrosis in schistosomiasis. We recently purified the granuloma-derived growth factor to homogeneity by a two-step procedure. When crude GS is subjected to gel filtration chromatography (Bio-Gel P-30), the fibroblast stimulating activity is retrieved in fractions with apparent Mr 20-25 kD ("P-30 peak"). When the "P-30 peak" is incubated with heparin-sepharose, the biological activity is retained on the matrix. This activity can be eluted from the matrix with 1.2-1.5 M NaCl. SDS-PAGE analysis of the eluate reveals a single band when stained with silver nitrate. Moreover, the heparin-binding protein elutes in a single peak when subjected to ion exchange FPLC. The purified fibroblast growth stimulating factor does not stimulate growth of endothelial cells, unlike members of class I and class II heparin-binding growth factors. Its biological activity is not neutralized by anti-PDGF antibody. In view of these distinctive biological and physical characteristics, we designate the granuloma-derived growth factor, "fibroblast-stimulating factor 1" (FsF-1).

- 197 INDUCTION OF T HELPER CELL HYPORESPONSIVENESS TO ANTIGEN BY MACROPHAGES FROM SCHISTOSOMAL EGG GRANULOMAS: A BASIS FOR IMMUNOMODULATION IN SCHISTOSOMIASIS?

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Immunomodulation in experimental murine schistosomiasis consists of predictable down-regulation of granulomatous hypersensitivity. However, it is still unclear what events precipitate the phenomenon, nor what cells or cell interactions carry it out. Testing the effect of granuloma macrophages (GM) from schistosomal egg granulomas on defined populations of lymphocytes, we found that they induced marked hyporesponsiveness to antigenic stimulation in T helper (TH) cells. Specifically, GM isolated from livers of infected C3H mice were cocultured with the I-E^k- restricted clone of TH type 1 (TH-1) cells AE7, together with the specific antigen fragment 81-104 from pigeon cytochrome c. When subsequently restimulated with antigen in the presence of splenic antigen presenting cells, the proliferative capacity of such TH-1 cells was inhibited by up to 90%, when compared to that of TH-1 cells precultured with either GM or antigen alone. The cells, however, responded readily to exogenous IL-2. Moreover, mab anti I-E^k--but not I-A^k--abrogated the induction of hyporesponsiveness. We surmise that increasing amounts of GM, in the presence of egg antigens, induce a similar hyporesponsive state in schistosome-specific subpopulations of T cells. This observation may explain the well known "suppressor" effects of GM on polyclonal T cell populations in schistosomiasis, as well as represent a major basis for the immunomodulation characteristic of this disease.

G: KINETOPLASTIDIA - IMMUNOLOGY

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A VIABLE POPULATION OF L3T4⁺ (CD4⁺) T LYMPHOCYTES IS NECESSARY FOR DEVELOPMENT OF RESISTANCE TO *Trypanosoma cruzi*.

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Immune mechanisms responsible for resistance to *T. cruzi* are poorly understood. Immunization with killed, chemically inactivated or with purified antigens of the organism induces partial protection. To examine the role of L3T4⁺ (CD4⁺) T lymphocytes in the of resistance to *T. cruzi*, we used the ability of mice to mount a partial resistance following immunization. Antigens from epimastigotes were used to immunize mice either alone (EpiAg) or with 30 µg of saponin (EpiAg-S). Each mouse received one weekly injection for 5 weeks. Monoclonal antibody GK1.5 (MAb) against the L3T4 cell surface glycoprotein of a subset of T lymphocytes was injected ip 3 days before each immunization. The results revealed that immunization with EpiAg only or with EpiAg-S resulted in a strong antibody response, particularly when S was used. In contrast, antibodies to EpiAg were not detected in mice treated with MAb and immunized with EpiAg or were at very low titer when EpiAg-S was used. Mice injected with MAb and immunized with EpiAg did not form antibodies to EpiAg, whereas mice immunized with EpiAg only or EpiAg-S formed antibodies which reacted with a number of polypeptides as shown by immunoblotting. Delayed-type hypersensitivity (DTH) was almost completely abrogated by use of MAb. Mice immunized with EpiAg developed a minor DTH reaction which was completely absent in mice injected with MAb and immunized similarly. In contrast, mice immunized with EpiAg-S developed a strong DTH reaction which was, however, considerably less evident in mice injected with MAb and immunized similarly. One week after the last immunization each mouse was challenged with 1x10⁵ bloodform trypanosomes. Mice immunized with EpiAg had 100 % mortality. In contrast, 50 % of the mice immunized with EpiAg-S were protected and survived. Development of resistance, however, was completely abrogated by injection of MAb; mice injected with MAb and immunized with EpiAg-S had 100% mortality.

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THE gp63 GENE OF *Leishmania donovani chagasi* LACKS THE RGD MACROPHAGE BINDING LIGAND. *R. A. Miller, M. Parsons, S. G. Reed. Seattle Biomedical Research Institute, Seattle, WA 98109.

The predominant surface protein on promastigotes of human pathogenic *Leishmania* spp. is an immunogenic glycoprotein of approximately 63 kD molecular mass. It is a zinc metalloprotease and in some *Leishmania* spp. may contribute to macrophage attachment. Two recombinant clones containing the gp63 gene were selected from a λgt11 genomic library of the new world parasite *L. donovani chagasi*. The *L. d. c.* genome contains at least 7 tandemly linked copies of the gp63 gene with a repeat unit length of 3.05 kB. Genomic Southern analysis suggests that there are restriction site polymorphisms in the repeats. The DNA sequence of the predicted coding region (1.8 kB) is 92% identical to the gp63 gene of *L. major*. Regions which are very strongly conserved are the carboxy terminus, the signal peptide domain at the amino terminus, and the consensus zinc-binding sequence. Three distinct one-base additions in the *L. d. c.* gene (when compared with the *L. major* gene) result in a frame shift abolishing homology over a span of 56 amino acid residues in the middle of the mature peptide. As a result the predicted protein sequences are only 77% homologous. The RGD sequence implicated in binding to the C3 receptor of macrophages, and which is present in the *L. major* sequence, lies within this region and is not present in the *L. d. c.* protein. The absence of this ligand group has potential implications for the interaction of *L. d. c.* promastigotes with host cells. Computer analysis of the predicted protein has identified probable T-cell and B-cell epitopes which will be useful for future immunologic studies, including work on serodiagnosis and vaccine development.

G: KINETOPLASTIDIA - IMMUNOLOGY

200 THE ROLE OF TUMOR NECROSIS FACTOR IN EXPERIMENTAL MURINE CUTANEOUS LEISHMANIASIS.

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This study investigates the ability of the cytokine tumor necrosis factor (TNF) to activate infected macrophages in vitro to destroy the intracellular parasite Leishmania major. Previous work from our laboratory demonstrated that the ability of inbred strains of mice to produce TNF in response to L. major directly correlated with their level of resistance to infection. Furthermore, administration of recombinant human TNF (rHuTNF) to resistant (C3H) or susceptible (BALB/c) strains of mice resulted not only in an impairment of lesion development but a decrease in the number of parasites recovered from the lesions or the draining lymph nodes of these animals as well. Injection of anti-TNF antibody into either strain of mice generated the opposite effect. In the present study, the in vitro treatment of L. major parasitized peritoneal exudate cells (PECs) with 100u/ml rHuTNF resulted in a decrease in the number of intracellular parasites observed. This effect of TNF could be enhanced by daily addition of rHuTNF to the culture system. Furthermore, this decrease in the intracellular parasite burden could be observed when PECs were treated with TNF either prior to or after infection with L. major. Ongoing studies are investigating whether the action of TNF on L. major is a parasitocidal or parasitostatic effect. In addition, the ability of TNF to respond synergistically with IFN-gamma is also being investigated.

201 PATTERN OF LYMPHOKINE SECRETION IN MURINE LEISHMANIASIS: CORRELATION WITH DISEASE PROGRESSION.

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Murine CD4⁺ T cells can be divided into 2 subsets: Th1 (inflammatory) cells which produce IL-2 and IFN-gamma and Th2 (helper) cells which produce IL-4, 5 and 6. In murine experimental cutaneous leishmaniasis induced with Leishmania major, different inbred mouse strains vary in their susceptibility to infection, and CD4⁺ L. major-specific T cells can either exacerbate or ameliorate the course of infection with the parasite. It is therefore possible that preferential activation of the Th1 or Th2 subsets influences the outcome of infection with L. major. We examined the lymphokines produced by BALB/c (susceptible) and B10.D2 (congenic with BALB/c, resistant) mice infected with L. major. Using cell lines responsive to various cytokines and specific blocking mAbs, it was found that whereas BALB/c mice produced predominantly IL-4, B10.D2 produced IL-2 in response to infection with the parasite. In addition, IFN-gamma production generally correlated with resistance. In experiments using reciprocal radiation chimeras made between BALB/c and B10.D2, it was found that the lymphokines produced by the chimeric mice in response to infection with L. major was determined by the donor cells. Finally, BALB/c mice were immunized against L. major by injection of chemically-mutagenized avirulent L. major and these mice were found to now produce primarily IL-2 and no longer IL-4 in response to infection with L. major. These results indicate that susceptibility to leishmaniasis in mice correlates with the lymphokines produced in response to infection and are in agreement with recent similar experiments of Heinzel, et al. (J. Exp. Med. 169:59, 1989) using L. major-infected mice and cytokine-specific molecular probes.

G: KINETOPLASTIDIA - IMMUNOLOGY

- 202 EVIDENCE FOR TWO DISTINCT PATHWAYS OF MACROPHAGE ACTIVATION FOR ANTILEISHMANIAL DEFENSE. J.P. Sypek* and D.J. Wyler, New England Medical Center Hospitals and Tufts University School of Medicine, Boston, MA 02111.

Antileishmanial defense has been ascribed to the antimicrobial effects induced by soluble macrophage activating lymphokines ("MAF's") such as interferon γ and GM-CSF. Comparison of two cloned murine hybridoma T cell lines that in vitro induce antileishmanial effects in *L. major*-infected macrophages provided evidence for an additional, lymphokine-independent, defense mechanism. One clone, 1D5, produced MAF's that induced antileishmanial effects; this production was suppressable by addition to culture of cyclosporine A (CsA), a drug that blocks the synthesis of MAF's. At 34°C, a temperature at which MAF's are relatively ineffective for antileishmanial defense, 1D5 failed to display its antileishmanial properties. In contrast, the other clone, 1B6, produced no MAF's; its ability to induce antileishmanial effects was not suppressable with CsA; and at 34°C it retained its ability to activate macrophage defense. In addition, antileishmanial defense induced by 1B6 but not by 1D5 was genetically restricted; infected macrophages and hybridomas had to be syngeneic for the effects to occur. Neither clone was cytotoxic to infected macrophages or produced detectable GM-CSF. These observations provide evidence for the existence of two distinct mechanisms of T cell-mediated antileishmanial defense: one that is MAF-dependent, and one that is apparently MAF-independent.

- 203 *Leishmania major* PROVIDES THE TRIGGER SIGNAL FOR MICROBICIDAL AND TUMORICIDAL EFFECTOR ACTIVITIES OF INTERFERON γ PRIMED MACROPHAGES. R.M. Crawford*, S.J. Green, M.S. Meltzer, C.A. Nacy. Walter Reed Army Inst. of Res., Washington, DC 20307-5100.

Macrophages are activated for microbicidal or tumoricidal activity by a two-stage signalling process referred to as priming and triggering. Interferon γ (IFN γ) is a major priming signal for macrophage cytotoxicity, but the nature of the trigger signal remains controversial. Endogenous trigger signals have not been characterized, and a physiological role for exogenous trigger signals like bacterial lipopolysaccharides (LPS) is difficult to explain in infections of other than bacterial etiology. We report that an L-arginine-dependent reactive nitrogen intermediate killing mechanism of activated macrophages is responsible for intracellular destruction of *L. major* amastigotes and extracellular destruction of Tu-5 tumor target cells. Macrophages treated with up to 100 U of IFN γ alone produced negligible amounts ($<1 \mu\text{M}$) of nitrite, a reactive intermediate in this effector pathway, and failed to kill tumor targets. Infection of IFN γ -treated macrophages with *L. major* amastigotes, however, resulted in a greater than 50-fold increase in nitrite production, with concomitant development of tumoricidal activity. Non-infective amastigotes (freeze-thawed, irradiated) and cultured promastigotes each induced nitrite production and tumoricidal activity in IFN γ -treated macrophages; in contrast, 1 $\mu\text{g/ml}$ of purified lipophosphoglycan (LPG) or 1 $\mu\text{g/ml}$ of gp63, both major promastigote surface molecules, did not. Polymixin B inhibited the production of nitrite and tumor cytotoxicity in IFN γ -treated macrophages triggered with 10 ng LPS, but had no effect on IFN γ -treated amastigote infected macrophages; amastigote preparations contained $<5\text{pg/ml}$ LPS by Limulus assay. These data suggest that *L. major* amastigotes, like LPS, can act as a trigger signal for both intracellular and extracellular killing activity of IFN γ -primed macrophages.

- 204 HUMAN T CELLS RECOGNIZE LEISHMANIAL ANTIGENS ON INFECTED MONOCYTES. P.C. Melby, G. Collet-Lima, and *D.L. Sacks. Laboratory of Parasitic Diseases, NIAID/NIH, Bethesda, MD.

Immunity to infection by the intracellular parasite *Leishmania* is mediated by sensitized T cells, however direct recognition of infected macrophages by T cells has not been clearly demonstrated. The ability of infected macrophages to directly activate T cells has been called into question because of the evidence in the murine model that there is significant down-regulation of IL-1 production and MHC antigen expression by infected macrophages. Experiments addressing this question have been confounded by the possibility that T cell activation in these systems might be due to release of leishmanial antigens by infected cells and subsequently presented by contaminating populations of uninfected antigen presenting cells (APCs) such as B cells or uninfected macrophages. We have addressed this issue using peripheral blood mononuclear cells (PBMNCs) from patients with healing cutaneous *L. major* infection. Monocytes were purified (>95%) from PBMNCs by use of a discontinuous Percoll gradient and then infected with serum opsonized *L. major* or *L. donovani* amastigotes. After 2 hrs free parasites were removed by washing and the infections allowed to proceed for 48-72 hrs, at which point over 90% of the cells were infected (2-4 amastigotes/cell). Cells were then lightly fixed with 0.32% paraformaldehyde to stop active infection and prevent further processing and possible release of parasite antigens. Addition of functionally pure autologous T cells resulted in strong proliferative responses to both *L. major* and *L. donovani* fixed infected monocytes (SI=9-143). This stimulation could not be augmented by the addition of uninfected monocytes. Supernatants from infected monocytes failed to stimulate unfractionated PBMNCs indicating the absence of non-cell associated soluble antigens. We conclude that *Leishmania* infected macrophages are able to efficiently process and present antigens to sensitized T cells.

- 205 HUMAN T-LYMPHOCYTE RESPONSES TO GP63, A MAJOR SURFACE ANTIGEN OF LEISHMANIA

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gp63, an abundant cell surface protein which appears to be highly conserved amongst leishmania, has been implicated in the ability of leishmania to infect macrophages in vitro and may have potential as a protective antigen in mice. However, very little is known regarding human immune responses to this glycoprotein antigen. We have evaluated human T-lymphocyte responses to native gp63 purified from *L. major* in individuals with active or cured cutaneous, mucosal and visceral leishmaniasis.

In vitro analysis of PBL obtained from patients recovered from *L. d. chagasi*, *L. d. donovani*, *L. b. brasiliensis* or *L. m. amazonensis* infections demonstrated strong proliferative responses in the *L. m. amazonensis* patients tested, but not in the other three groups. This suggests that certain species of leishmania share gp63-specific T-epitopes and that other species may contain non-crossreactive epitopes. In order to further evaluate these responses, gp63-specific lines and clones were established in continuous culture from the PBL of one of the individuals recovered from cutaneous leishmaniasis. Antigen-induced lymphokines, including IL-2, IFN- γ , and IL-4 profiles were assessed by bioassay and Northern blot analysis. In addition, we determined that this line responded to recombinant as well as native gp63. This has verified the potential of recombinant gp63 for evaluation of lymphocyte responses and may suggest its future usefulness as a diagnostic or immunoprophylactic antigen.

Intradermal skin testing was performed in patients with active mucosal disease or with cured visceral leishmaniasis. Positive skin reactions were elicited with native gp63 in 3 of 3 mucosal patients tested, while all post-kalazar patients were skin test negative. Surprisingly, PBL obtained from skin test positive individuals with mucosal disease did not proliferate in vitro in response to stimulation with native gp63. These results suggest that while in vitro assays provide valuable preliminary information, more extensive analysis in vivo may also be required in order to definitively characterize immune responses to specific antigens.

H: VIRAL PATHOGENESIS

IMMUNITY TO DEN (DEN) PROTECTS AGAINST DEATH DUE TO JAPANESE
206 ENCEPHALITIS (JE) B.L. Innis, A. Nisalak, C.H. Hoke, S. Suntayakorn,
P. Puttisri, V. Chongswasdi, C. Kaemkosolsri, N. Mimpitakpongs and P.
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Bangkok and the Ministry of Public Health, Thailand.

Infection by DEN virus or by JE virus accounts for essentially all human flavivirus infections in Thailand. Patients with JE who mount an anamnestic antibody response presumably are not. Earlier workers noted a probable difference in JE fatality rates between patients responding with a primary response compared to those with a secondary (anamnestic) response. We sought to expand on those findings. From 1985-8, 192 patients hospitalized in N.Thailand during the rainy season were diagnosed to have JE by detection of anti-JE IgM in CSF (n=186) or by detection of elevated serum anti-JE IgM associated with an altered mental state and a CSF pleocytosis (n=6). The outcome of JE was known in 174 cases, all but 3 of which could be classified as either primary or secondary JE. Criteria for this classification were set while blinded to outcome by examining the frequency distribution of the ratio of anti-JE IgM to IgG (ELISA units) for 66 paired sera categorized as primary or secondary by the results of anti-JE/anti-DEN hemagglutination-inhibition assay. The fatality rate among those with primary JE (15/51; 95CI 17-44%) was 3.2 times that among those with secondary JE (11/120; 95CI 5-16%). The age distributions for both groups were similar. Differences in age did not account for the marked difference in survival. These data show that DEN immunity protects against death from JE. In regions of SE Asia where costs preclude universal JE immunization, the youngest children, who are likely to be DEN non-immune, should be selected for priority receipt of vaccine.

BIOLOGICAL MECHANISM RELATED TO VIRULENCE OF DENGUE-2 VIRUS.
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Our previous studies suggest that the virulence of dengue-2 virus was related to the ability of virus to infect freshly isolated human monocytes in the presence of enhancing antibodies. To investigate the mechanism for dengue-2 virulence, viral binding capacities to monocytes were compared for two pairs of dengue-2 strains exhibiting high and low infectivity, from two different geographic locations. No difference was observed in the binding efficiencies of viral strains exhibiting high and low monocyte-infectivity, even though the eventual viral titers produced in cells differed by 8-12 fold. The rate of viral internalization appeared to be comparable between the members of each viral pair. However, strains with high-monocyte-infectivity exhibited a wider pH range and required a more basic pH for viral membrane fusion than did strains with low monocyte-infectivity. In addition, viral strains with low infectivity exhibited a higher sensitivity to infection-inhibition by chloroquine than did the strains with high infectivity. These findings suggest that difference in viral uncoating rather than attachment, may be associated with monocyte-infectivity and thus related to virulence of dengue-2 virus.

H: VIRAL PATHOGENESIS

- 208 BUNYAVIRUS-VECTOR INFECTION MODEL: MONOCLONAL ANTIBODIES DIRECTED AGAINST THE G2 GLYCOPROTEIN OF LA CROSSE VIRUS. *G.V. Ludwig, T.M. Yuill, B.M. Christensen, and K.T. Schultz University of Wisconsin-Madison, Madison, WI.

We recently reported a possible model for attachment and infection of vector midgut tissues by bunyaviruses. More specifically, we have shown that enzymatic modification of La Crosse virus may be required for attachment of virus to mosquito midgut cells. Confirmation of our model, and identification of the virus attachment protein required the production and characterization of monoclonal antibodies directed against the G2 glycoprotein of La Crosse virus. Hybridomas were produced utilizing standard myeloma cell/B lymphocyte fusion techniques. The hybridoma cell lines were screened for their ability to precipitate the 35 kDalton G2 glycoprotein of La Crosse virus from ³⁵S-cysteine labeled virus lysate. Positive testing cell lines were cloned by limiting dilution. Monoclonal antibodies from positive hybridomas were isotyped, and tested for their ability to neutralize La Crosse virus under a variety of conditions. G2, purified by affinity chromatography, was used in virus attachment inhibition studies to help identify the role of G2 in virus attachment. Data presented tend to support our model, and further suggest that G2 may be the virus attachment protein for La Crosse virus in mosquitoes.

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DUGBE VIRUS SUSCEPTIBILITY TO NEUTRALISATION BY MONOCLONAL ANTIBODIES AS A MARKER IN MICE.

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Monoclonal antibodies (Mab) against the envelope protein of Dugbe virus were compared for their ability to neutralise seven different strains of Dugbe virus. The tests identified essentially two groups of virus viz. Those that were readily neutralised and those that were either poorly or not neutralised. All Mab were shown by indirect immunofluorescence tests to bind to all viruses.

Comparisons of the neurovirulence of these viruses for mice showed a correlation with the capacity to be neutralised, in that the most virulent (assessed on the basis of survival time) were the most poorly neutralised. This was also reflected in mouse protection experiments, when Mab were administered passively to mice at least three weeks old.

Our results imply that the presentation of the epitope is an important factor in determining virus virulence.

H: VIRAL PATHOGENESIS

210 COMPARISON OF THOGOTO VIRUS INFECTION IN A PERMISSIVE AND APPARENTLY NON-PERMISSIVE VERTEBRATE.

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Thogoto (THO) virus is a tick-borne arbovirus which shares structural and morphogenetic similarities with the influenza viruses (family, Orthomyxoviridae). In the laboratory, hamsters are susceptible hosts of THO virus and develop high viremic titers, whereas guinea pigs do not develop a detectable viremia (< 20 PFU/ml blood). However, when infected (Donor) and uninfected (Recipient) *Rhipicephalus appendiculatus* ticks (a biological vector of THO virus) co-feed on guinea pigs, a high percentage of recipient ticks acquire virus (non-viremic transmission). A factor(s) associated with the salivary glands of ticks potentiates non-viraemic transmission (NVT).

To investigate the pathogenesis of THO virus in hamsters, a 400 nucleotide RNA biotinylated probe complementary to segment 3 of THO virus was constructed for detection of viral RNA by *in situ* assays on cryostat sections of selected tissues. Antigen in tissue was detected using the indirect immunofluorescent antibody staining technique. Thogoto viral RNA was detected in brain, heart, liver, spleen and kidney at 3 days post-inoculation, but was not detected in the lung, axillary lymph nodes or ovaries. Using immunogold labelling techniques, THO virus also appeared to be associated with the leukocytes. Using the above techniques, studies are at present being conducted in guinea pigs, and infected leukocytes from hamsters are being characterised.

TRANSMISSION OF HEPATITIS E VIRUS TO OWL MONKEYS (*AOTUS TRIVIRGATUS*).

211 *J. Ticehurst, L. Rhodes, K. Krawczynski, L. Asher, W. Engler, J.

Caudill, M. Sjogren, C. Hoke, J. LeDuc, D. Bradley, L. Binn. Walter Reed Army Inst, Washington DC; US Army Med Res Inst Infect Dis, Frederick MD; Ctrs Dis Control, Atlanta, GA; Armed Forces Inst Path, Washington DC.

It has been difficult to establish animal models of infection with hepatitis E virus (HEV), the agent of enterically-transmitted non-A, non-B hepatitis¹. Among several susceptible species of primates, cynomolgus monkeys have been the most useful². Efforts to understand the virus and its epidemiology have been hampered by limited amounts of HEV and by low concentrations of anti-HEV in most convalescent-phase sera. We inoculated 6 owl monkeys with feces from Mexico known to contain infectious HEV. All seroconverted and had high levels of anti-HEV 6 months after inoculation, detected by immune electron microscopy. Three had biochemical and histopathologic evidence of hepatitis, but HEV was not detected by immunofluorescence analysis of liver tissue from 2 of 4 biopsied owl monkeys. (All 5 cynomolgus monkeys given the same inoculum developed hepatitis; one, studied in detail, seroconverted, had HEV antigen in liver, and excreted HEV particles into bile and feces.) Although these owl monkeys did not excrete detectable HEV or uniformly develop hepatitis, all were infected and developed sustained high-level antibody responses that may be valuable for understanding immunity to HEV and for developing rapid immunoassays.

1. Purcell RH, Ticehurst JR. In Zuckerman A (ed): Viral Hepatitis and Liver Disease. Alan R Liss, 1988, p 131-7;

2. Bradley DW, Krawczynski K, Cook EH Jr, et al. Proc Natl Acad Sci USA 1987; 84:6277-6281

I: MALARIA - MOLECULAR BIOLOGY

212 A GENE THAT ENCODES FOR AN ERYTHROCYTE BINDING PROTEIN OF PLASMODIUM KNOWLESI MEROZOITES.

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Human erythrocytes that lack Duffy blood group antigens are refractory to invasion by Plasmodium knowlesi and P. vivax merozoites. Recently, a malaria protein of 135 kDa from culture supernatants was identified that specifically binds the Duffy blood group antigens (Haynes et al.(1988) J. Exp. Med. 167:1873-1881). Monospecific polyclonal antibodies were affinity purified from immune rhesus sera against this 135 kDa Duffy-binding parasite protein and were used to screen a late schizont cDNA lambda gt11 expression library of P. knowlesi. Two cDNA clones were identified that hybridized with each other and with a 4.5 kb message from late schizont RNA. These cDNA clones hybridized to chromosomes of 3 sizes (1.2×10^6 , 1.8×10^6 , 3.6×10^6). The translated cDNA sequences have a predicted 22 amino acid transmembrane segment followed by 48 residues at the C-terminus. Antibodies selected by plaque lifts of each cDNA clone immunoprecipitated from metabolically-labeled cultures the 135 kDa P. knowlesi Duffy binding protein and an additional protein, of approximately 150 kDa, from Triton X-100 solubilized merozoites. In pulse-chase experiments, the 150 kDa protein present in Triton X-100-solubilized merozoites was converted to a culture supernatant protein of identical mobility to the 135 kDa Duffy binding protein. These data suggest that the Duffy binding protein on the P. knowlesi merozoite is an integral membrane protein and that it is cleaved during culture to release a 135 kDa protein into the supernatant. This is the first step in exploring the molecular biology of the Duffy binding protein and the family of genes involved in invasion of P. knowlesi merozoites into red blood cells.

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HEAT SHOCK PROTEINS OF PLASMODIUM BERGHEI AND P. FALCIPARUM SPOOROZOITES AND EXOERYTHROCYTIC PARASITES.

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Heat shock proteins (hsp) and the 78 kDa glucose-regulated proteins (grp78) have been described in eukaryote cells and are encoded by multigene families. Hsp are localized in the cytoplasm and nucleus, and may be either expressed constitutively or induced by environmental stress. grp78 have a signal sequence and remain in the lumen of endoplasmic reticulum (ER). Genes for similar proteins in P. falciparum have been cloned. Interestingly, Pfgrp contains an amino acid sequence that resembles the putative hepatocyte binding region of CS protein. Sera to Pfgrp and Pfgrp were tested by immuno-gold electron microscopy with sporozoites and exoerythrocytic (EE) parasites and western blots with sporozoites. Pfgrp was not found in the sporozoites, but was detected in newly invaded sporozoites and in the nuclei and cytoplasm of EE parasites. Pfgrp was detected in the ER of sporozoites and EE parasites. These results suggest that heat shock proteins of P. falciparum and P. berghei are conserved, and that these genes may play a role in the development of these parasites in the invertebrate vector and the vertebrate host. (Supported by funds from NIH, USAID, WHO and MacArthur Foundation).

I: MALARIA - MOLECULAR BIOLOGY

- 214 USE OF THE POLYMERASE CHAIN REACTION (PCR) TO AMPLIFY MALARIA DNA AS A TARGET FOR DNA PROBES. R.H. Barker jr* and D.F. Wirth. Harvard School of Public Health, Boston, MA, USA.

We have previously reported on studies developing methods for application of DNA probes as epidemiological tools in malaria research. In the present study we examine factors involved in adapting the PCR technique for amplification of malaria DNA in field samples. Using purified *P. falciparum* DNA, target sequences were amplified at least 10^5 -fold; using crude samples prepared both as liquid and dried samples we obtain at least 10^3 -fold amplification. This approach can significantly increase the sensitivity of DNA probe based diagnostic methods, facilitating use of nonradiolabelled probes, and permitting diagnostic use of DNA probes whose nucleotide sequences are not highly repeated in the parasite. This in turn will permit use of probes having more limited specificity, such as use of isolate-specific probes for the S antigens.

- 215 MAPPING B EPITOPES OF THE PLASMODIUM FALCIPARUM PFS 25 OOKINETE PROTEIN AND THE PRODUCTION OF SYNTHETIC PEPTIDE ANTIBODIES REACTIVE TO THE NATIVE PROTEIN: IMPLICATIONS FOR VACCINE DEVELOPMENT

I.A. Quakyi, D.C. Kaslow, M.F. Good, S.N. Isaacs, J.H. Nunberg, J.A. Berzofsky, B. Moss, R. Houghton, D.P. Keister, L.H. Miller. National Institutes of Health, Bethesda, MD, Cetus Corporation, Emeryville, CA, Scripps Clinic and Research Foundation, La Jolla, CA.

The 25kDa surface antigen of *Plasmodium falciparum* zygotes and ookinetes (Pfs 25) has been previously cloned and sequenced. Pfs 25 is cysteine rich, contains 4 tandem EGF-like domains. The epitopes of Pfs 25 recognized by monoclonal antibodies that block transmission are destroyed by reduction, indicating that secondary structure is important for functional activity. To map and characterize B epitopes that may be crucial for the development of a peptide vaccine as an alternative strategy to recombinant Pfs 25 vaccine, we raised antisera to 41 overlapping peptides (each peptide is approximately 15 amino acids in length) using recombinant interleukin 2 in Freund's complete adjuvant. Fifty-four per cent of these peptides elicited an antibody response. By western blot analysis of native and reduced Pfs25, we identified 8 B epitopes, antibodies to 5 of which recognize native protein and 3 reduced protein. We have also identified anti-peptide antibodies that recognize the surface protein of live parasites. MAb 1C7 reacts with the surface proteins of live parasites by immunofluorescence, and agglutinates them, and maps to the first EGF-like domain of Pfs25. Interestingly, the anti-peptide antiserum that reacts most strongly to the surface of live parasites, and agglutinates them, also recognizes the same predicted loop structure as MAb 1C7, but on a different EGF-like domain of Pfs25. Although epitopes in cysteine-rich proteins are destroyed by reduction, peptides to these epitopes can induce antibodies which can bind to native-like conformational structure.

I: MALARIA - MOLECULAR BIOLOGY

216 USE OF SYNTHETIC DNA OLIGOMERS TO ANALYZE THE REPETITIVE DAN FAMILIES OF *PLASMODIUM FALCIPARUM*.

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A degenerate 21-oligonucleotide long family of repetitive DNA is the target for most candidate DAN hybridization probes for the diagnosis of falciparum malaria. The original selection of the nonisotopic probe, PFR1-AP, was based on relatively few sequences (Franzen et al, 1984, Lancet p. 525). About 7 additional repetitive sequence families in the *P. falciparum* genome have now been defined by Drs. Petterson, Lizardi, McCutchan, Scaife, Zolg, Mons, Viadya, Guntaka, Weber, and coworkers. Using Southern analysis and dot-blot hybridization of genomic falciparum DNA, restriction fragment patterns were defined, and the relative abundance of these repetitive families were estimated, after hybridization with labeled 21-base long synthetic oligomers derived from each sequence family. Typically, distinctive fingerprint patterns were obtained with each repetitive sequence family. However, several repetitive oligomers hybridized with a number of fragments of the same molecular weights after Sau 3A or Hind III digestion. Most strikingly, after Dra I digestion, all detectable 21-mer sequence family fragments were of high molecular weight. These and additional results indicate that repetitive families of *P. falciparum* are sometimes linked at many chromosomal locations in the *P. falciparum* genome. Linkage of repetitive families may have implications regarding PCR amplification, the uniformity of some plasmid-borne probes, and capture-detection procedures. An in-frame consensus sequence of the 21-base long family (5' ACTAACATAGGTCTTAACCTG) was the most sensitive of the oligomers tested. Supported in part by DiaTech subagreement #86110160 and by funds from the World Health Organization.

217 CLONING, SEQUENCING AND EXPRESSION OF A 17 AMINO ACID REPEAT ANTIGEN GENE FROM *PLASMODIUM FALCIPARUM* EE PARASITES. J. Zhu, B. Sina, K. Saknuja, A. Lal, M. R. Hollingdale. Department of Malaria, Biomedical Research Institute, Rockville, MD.

The exoerythrocytic (EE) stages of malaria parasites have been shown to contain both sporozoite CS proteins, and antigens common to red blood stages. Only recently was a *Plasmodium falciparum* EE stage-specific antigen described, Guerin-Marchand et al. 1987, who published three repeats of a 17 amino acid sequence. We have synthesized peptide vaccines containing the repeat sequence and described immunogenicity and stage specificity properties of this antigen. Here we report the isolation and expression of a 1.6kb DNA sequence from *P. falciparum* 7G8 using an oligonucleotide probe spanning part of the repeat. Sequence analysis revealed that it consists of 21 tandem major repeats of the 17 amino acid sequence EQQSDLEQERLAKEKLQ interrupted by 3 minor repeats of the 17 amino acid sequence EQQSDLERTKASTETLQ. Certain polymorphic changes of amino acids within either repeat have been observed. The 1.6kb DNA fragment was inserted into pGEM 7Zf vector and expressed as a LacZ fusion protein under β -galactosidase promoter. The expressed protein was recognized by antisera against the 17 amino acid major repeat. The structure of the DNA sequence and the putative open reading sequence and/or introns will be discussed.

I: MALARIA - MOLECULAR BIOLOGY

218 IMMUNOGENICITY OF PLASMODIUM FALCIPARUM AND VIVAX CIRCUMSPOROZOITE PROTEINS, AND THEIR ANALOGS, PRODUCED IN YEAST.

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The yeast Saccharomyces cerevisiae has been shown to be an excellent host system for the production of malaria circumsporozoite (CS) proteins. High yielding intracellular systems, and secretion strategies based on the yeast α -factor mating pheromone system have allowed the production of many derivatives of Plasmodium falciparum and vivax CS proteins. In addition, the extreme hydrophilicity of these molecules has allowed their facile purification to a standard suitable for immunogenicity studies in animal model systems. Immunogens containing the characteristic CS protein repeat units flanked by regions containing postulated T cell epitopes have been expressed at high levels. Similarly, synthetic genes encoding the flanking regions and only two or four of the repeat units have given high yields of CS protein analogs. The purified proteins have been assessed for their potential in the immunoprophylaxis of malaria in a variety of animal models using several types of adjuvant systems. Several parameters have been used to measure responses to immunogen formulations with both alum and muramyl tripeptide adjuvants. Sporozoite challenge studies with a P. vivax CS protein in Saimiri monkeys have led to the introduction of a P. vivax CS protein/alum formulation into human clinical trials.

219 EXPRESSION IN YEAST OF Pfs25, A SEXUAL STAGE ANTIGEN OF PLASMODIUM FALCIPARUM.

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Transmission of Plasmodium falciparum, the major human malaria, can be blocked by antibodies against sexual stage-specific antigens. Pfs25 is a surface antigen of zygotes and ookinetes of P. falciparum. Recombinant DNA-derived Pfs25 proteins represent candidate vaccines for the development of transmission blocking immunity in inhabitants of endemic areas. The gene for Pfs25 encodes a region with four regions of significant homology to epidermal growth factor (EGF). These regions are flanked by putative signal and anchor sequences. We have previously reported the high level secretion and accurate processing of human EGF from Saccharomyces cerevisiae using the yeast α -factor leader sequence. Accordingly, using synthetic DNA, we have engineered various regions of Pfs25, and glycosylation site mutants of Pfs25, for expression using this system. The availability of large quantities of recombinant Pfs25 derivatives now allows analysis of their immunogenicity in animal model systems, and also a comparative assessment of the relationship of Pfs25 to mammalian EGFs.

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EXCYSTATION OF IN VITRO DERIVED GIARDIA LAMBLIA CYSTS.

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In order to complete the life cycle of Giardia lamblia in vitro and to subject in vitro derived cysts to the most stringent test of biological activity, we measured their ability to excyst under differing conditions. The percent excystation (2-9%, calculated by the formula of Bingham et al., Exp. Parasitol. 47:284, 1979) was in the range reported for fecal cysts when water-resistant in vitro derived cysts (Gillin et al., Exp. Parasitol, in press) were exposed to the procedure of Schupp et al. (Gastroent. 95:1, 1988), but >80% of the emergent parasites were only partially excysted. In contrast, modifying the excystation procedure of Rice and Schaeffer (J. Clin. Micro. 14:709, 1981) increased excystation to 10-30% and complete emergence to >90%. In an attempt to identify the critical elements of excystation, we varied the emergence step of Rice and Schaeffer's protocol. Emergence was greatly (~10-fold) stimulated by exposure of in vitro cysts to certain proteases. Chymotrypsin (CT), trypsin (T), Proteinase K, Pronase E, subtilisin, and thermolysin were all effective, while pepsin and carboxypeptidases A and B were not. Heating or specific inhibitors destroyed the stimulatory activity of CT and T. Although the walls of in vitro cysts were visibly partially digested by protease treatment, trophozoites emerged only from one pole, as observed with fecal cysts. These studies have shown that the life cycle of G. lamblia can be completed in vitro and should augment our understanding of excystation.

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Restricted Variant Surface Epitopes on Giardia

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Giardia lamblia undergoes surface antigenic variation. The number of variant surface antigens and whether different isolates are capable of expressing the same antigenic variants are not known. The presence of certain epitopes on the surfaces of trophozoites from different isolates and clones was determined using 4 surface reacting Mabs to variants derived from WB(Mabs 6E7, 5C1, and 3F6) and GS/M(G10/4). In order to detect small numbers of reactive trophozoites, a 100,000 viable trophozoites per test were screened by IFA at 4 degrees C. Of 27 isolates, 10 possessed trophozoites reactive with Mabs 6E7, 5C1, and 3F6, 6 with Mab 3F6, 2 with G10/4, 1 with 5C1, 1 with 6E7, and 7 showed no reactivity. To exclude contamination, newly established clones derived from different isolates were also screened and were found to generate small numbers of reactive trophozoites similar to their parents. Generation of reactive trophozoites occurred approximately every 14 generations. By using Mabs conjugated with fluorescein or rhodamine, only one epitope was found on any single trophozoite. Analysis of the surface antigens of Mab 6E7 reactive clones from 3 isolates (CAT-1, Be-2, and G3M) revealed that Mab 6E7 reacted with surface antigens of different molecular masses. The variant antigenic epitopes present on Giardia are isolate dependent and can be mutually exclusive. The antigenic repertoire of isolates differs and this may have important biological implications.

J: GIARDIASIS AND TOXOPLASMOSIS

- 222 IDENTIFICATION OF MULTIPLE GIARDIA LAMBLIA ANTIGENS FROM HUMAN STOOL.
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The availability of sensitive assay methods has made it possible to diagnose parasitic infections by detection of parasite antigens in host materials. In the course of developing reagents for use in an antigen-capture test system, we have identified three distinct Giardia antigens occurring in the stools of infected patients. Stools from infected or control patients were emulsified in saline and clarified by centrifugation. Antigens were identified in Western Blots by probing with anti-Giardia cyst serum prepared in rabbits. Later we employed antisera prepared against Giardia antigens purified from the feces of infected gerbils. These sera were used to detect an antigen with an apparent molecular weight of 65 KD in a human stool extract which appears to be the same as a 65 KD antigen detected in the membranes of Giardia trophozoites. Subsequently, we have detected antigens of 78 KD and 82KD in the stools of different patients. To date, no patient has been found to have more than one antigen, and none of these antigens have been found in the stool of persons not infected with Giardia. We have produced antisera to the 78KD and the 82 KD molecules purified from human stools and these antisera react with each of the three antigens. We have tested human stools containing the 65KD, the 78KD and the 82KD antigens with the Alexon Prospect Giardia Assay and all have been reactive. This assay detects a 65 KD Giardia antigen, but we have no direct evidence that this test measures the same 65 KD molecule that we describe here. Based on our studies to date, it is not clear whether the set of antigens we describe represent strain markers which characterize Giardia strains or whether the antigens are derived from a Giardia protein and are modified in the intestinal milieu.

- 223 SEROTYPING OF GEOGRAPHICALLY DISTINCT HUMAN GIARDIA LAMBLIA
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Center for Tropical Diseases, Lowell, MA

We have previously reported the optimum electrophoretic and electroblotting conditions for the separation of Giardia lamblia membrane antigens. In the present investigation we have used these adaptations to study the cross-reactivity between isolates from Afghanistan, Egypt, the Sudan and the United States. The membrane antigens were separated on a 5-15% gradient SDS-PAGE in the presence of thioglycolic acid (TGA) and electroblotted to a zeta-probe membrane. Membranes were incubated with rabbit antisera to specific isolates or with antisera from human giardiasis patients, and visualized with a peroxidase conjugated antisera. The findings demonstrate considerable heterogeneity in membrane antigens of the G. lamblia isolates studied. The differences detected by the two antisera type parallel each other and allowed for grouping of the 12 isolates into 3 major groups according to their degrees of cross-reactivity and number of reactive components. Moreover under the experimental conditions, used, we could identify in addition to the several minor antigens at least twelve major antigens which differ in their frequency of appearance in the different groups. Preliminary experiments also indicate that there may also be quantitative differences between the isolates in some of these antigens. Furthermore, other isolate, as well as, group specific fractions were well resolved which could be potential typing markers. Based on the results obtained using a limited number of patient sera, it may be difficult to identify a single universal antigen for the routine immuno diagnosis of giardiasis. Data from this study also support previous findings of heterogeneity amongst isolates not only from widely separated geographic areas but also within a single region.

J: GIARDIASIS AND TOXOPLASMOSIS

224 CHARACTERIZATION AND cDNA CLONING OF A 22 kDa SURFACE ANTIGEN (p22) OF *TOXOPLASMA GONDII* (Tg).

*J.B. Prince, J. Huskinson, K.L. Auer, and J.S. Remington. Research Institute, Palo Alto Medical Foundation, Palo Alto, CA and Stanford University School of Medicine, Stanford, CA.

Our efforts to develop a subunit vaccine against Tg, in addition to our search for improved methods for diagnosis of Tg infection, have prompted us to clone and express the gene encoding p22, a major membrane antigen located on the surface of Tg tachyzoites (Tgt). Monoclonal antibodies (MAbs) 5A6 and 6D10, which were raised against a membrane-enriched fraction of Tg RH strain and which recognize a single antigen with an apparent size of 22 kDa in Western blots of reduced or unreduced Tgt, are both positive in the Sabin-Feldman dye test (for complement-fixing Abs) and the immunofluorescent antibody test using live Tgt. These data suggest that both MAbs recognize p22 on the parasite surface. When these MAbs were used to screen a Tgt cDNA expression library in λ gt11, two overlapping clones were obtained that together encompass 1055 bp of cDNA sequence. The recombinant fusion polypeptides produced by the two clones reacted specifically on Western blots with both of the MAbs and with IgG antibodies in sera from humans infected with Tg. The cloned composite cDNA sequence contains a 5' open reading frame of 510 bp (encoding a polypeptide with MW of 17,364) and a 14-bp poly(A) tail at the 3' end. Primer extension analysis revealed that the 5' end of the mRNA is about 300 bp upstream of this composite cDNA sequence. Consistent with the latter result, the cloned cDNAs hybridize in Northern blots to a single RNA band about 1.5 kb in size. The putative polypeptide contains no N-linked glycosylation sites but has a hydrophobic C-terminus characteristic of surface proteins that possess a glycolipid anchor. Experiments are in progress to clone and sequence the remaining 300 bp at the 5' end of the cDNA and to determine whether this antigen is in fact anchored to the surface by a glycolipid.

225 A REPRODUCTIVELY DEFICIENT MUTANT TOXOPLASMA VACCINE FOR CATS. J.K. Frenkel*, E.R. Pfefferkorn, J.L. Fishback and D.D. Smith Department of Pathology, University of Kansas School of Medicine, Kansas City, KS and Department of Microbiology, Dartmouth Medical School, Hanover, NH.

Kittens were immunized against oocyst shedding after normal bradyzoite infections, and even if treated prophylactically with monensin, which inhibited oocyst shedding. However, infection with bradyzoites of an oocystless toxoplasma strain did not immunize. These observations suggested that immunization might depend on stage-specific antigens produced during part of the enteric cycle, while the whole cycle was not necessary. We therefore decided to search for a reproductively deficient strain of toxoplasma that immunized without being accompanied by oocyst shedding. Clones of toxoplasma, mutagenized with a nitrosoguanidine compound (Pfefferkorn and Pfefferkorn 1979) and that had been selected for resistance to adenine arabinoside, were maintained in mice as chronic infections. 117 such clones were tested in cats by feeding them mouse brains containing bradyzoites. Most infections gave rise to oocyst shedding, except for infection with ten mutants. Of these, six mutants did not confer immunity and three were lost. One mutant, T-263, was tested in thirty-seven cats, none of which shed oocysts; when challenged, 31 or 83.7% were immune. A similar percentage had been immunized after monensin-controlled infection. Immunization with T-263 would be more practical than with a monensin-treated infection, because vaccine strain infected mice could be used for delivery. By curbing oocyst shedding this altruistic vaccine could markedly reduce the transmission of toxoplasma. (Supported by NIH grant AI-23730).

K: TROPICAL VETERINARY MEDICINE

- 226 T-LYMPHOCYTE PROLIFERATIVE RESPONSES AND CORRELATION OF CROSS-PROTECTION AGAINST VIRULENT RICKETTSIA RICKETTSII BY IMMUNIZATION WITH RICKETTSIA RHIPICEPHALI. K. L. Gage, T. R. Jerrells, and *D. H. Walker. University of Texas Medical Branch, Galveston, TX.

Rickettsia rhipicephali has not been reported to cause human disease but does induce a febrile disease in guinea pigs (GPs). Others have stated that GPs infected with this rickettsia are partially protected against challenge by R. rickettsii as determined by reduced mortality of immunized animals. Our study sought to confirm that infection of GPs with R. rhipicephali provides protection against R. rickettsii and tested the hypothesis that it has antigens that stimulate cross-protective T-cells. Groups of GPs (7 GPs/group) were infected with varying doses of R. rhipicephali (7.5×10^2 , 7.5×10^4 or 7.5×10^6 PFU's/GP). An additional 7 GP's were maintained as uninfected controls. Serum samples were collected from GPs 37 days later and 3 of the 7 GPs in each group were sacrificed to collect spleen cells for a lymphocyte proliferation assay. Analysis of the sera by indirect immunofluorescence demonstrated that titers against R. rhipicephali were 2- to 8-fold higher than against R. rickettsii. Antibody responses differed from those for the lymphocyte proliferation assay because both homologous and heterologous antigen strongly stimulated lymphocyte proliferation in a manner that corresponded to the number of R. rhipicephali contained in the immunizing dose. Both homologous and heterologous antigen stimulated lymphocyte proliferation equally well. The remaining 4 GPs from each group were challenged with 1.0×10^3 PFU's of R. rickettsii. The data indicate that R. rhipicephali protected GPs from fatal infection with R. rickettsii. Fever was not observed in challenged GPs immunized with the highest dose of R. rhipicephali and was a shorter duration in the other 2 immune groups compared to non-immune controls.

- 227 ANTIGENIC ANALYSIS OF EHRlichia CANIS AT THE POLYPEPTIDE LEVEL: IDENTIFICATION OF SPECIES-SPECIFIC ANTIGENS FOR USE IN THE DIAGNOSIS OF CANINE EHRlichIOSIS. S.J. Ma, *C.J. Holland, I. Kakoma, N. Rajapakse, and M. Ristic. University of Illinois, College of Veterinary Medicine, Department of Veterinary Pathobiology, Urbana, IL 61801.

Canine ehrlichiosis is a severe, often fatal tick-borne disease of dogs caused by the rickettsia Ehrlichia canis. Thus far, the only existing confirmatory serodiagnostic assay is the IFA test which is available in a very limited number of laboratories primarily in the U.S. Clearly, there is a need for a more simple, rapid, sensitive and specific serodiagnostic test suitable for field diagnosis of the disease. In this study, culture-derived E. canis was purified and subjected to SDS-PAGE and Western immunoblot analyses. Two species-specific E. canis polypeptides (70 kd and 35 kd) were selectively identified and isolated from SDS-PAGE gels by electroelution. N-terminal amino acid sequencing of these 2 purified polypeptides is in progress. The suitability of these polypeptides for use in a DOT-ELISA was studied. It was demonstrated that the 70 kd and 35 kd polypeptide consistently reacted with sera obtained from experimentally infected dogs beginning on days 9 and 14 post-inoculation, respectively. The results with sera of naturally infected dogs will be discussed.

K: TROPICAL VETERINARY MEDICINE

- 228 INTRACELLULAR MICROORGANISMS IN *DIROFILARIA IMMITIS*
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San Juan, Puerto Rico.

The observation of McLaren and co-workers of the presence of microorganisms in filariid larvae stimulated us to initiate a systematic study of other members of the Superfamily Filarioidea for the presence of similar organisms, and to compare the organisms found in each filariid host. The present study was undertaken to elucidate the morphology and the distribution of these organisms within the adult and the larval stages of *Dirofilaria immitis*. Sections of material prepared by standard methods for electron microscopy revealed that the distribution of the organisms within *D. immitis* is similar to that observed in *Onchocerca volvulus* and *Brugia malayi*: the microorganisms appear to be limited to the hypodermal cytoplasm of the lateral chords in the adults and in all larval stages, in the females they are also found in the rachis and the oocytes. The microorganisms of *D. immitis* and of other filariae share the following characteristics: Transovarian transmission; reproduction by binary fission and apparently also by a developmental cycle, resembling that of *Chlamydiae*, which consists of three developmental stages; and the location of organisms within cytoplasmic vesicles. The organisms of *D. immitis* appear to be larger and more robust than those of *O. volvulus*, *B. malayi*, and *L. carinii*, and frequently are present in greater numbers in the oocytes of *D. immitis* than are the organisms of other filariae. Further studies are in progress to elucidate the role(s) of these organisms in the development and survival of their nematode hosts.

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*DOT-ELISA FOR SERODIAGNOSIS OF ANAPLASMOSIS AND BABESIOSIS.

- 229 S. Montengro-James, A. T. Guillen, S.-J. Ma, P. Tapang, and M. Ristic.
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An ideal diagnostic assay should be sensitive, specific, reproducible, inexpensive and simple to perform. DOT-ELISA is a rapid, visually read enzyme immunoassay that meets with those requirements and now is widely accepted for the serodiagnosis of parasitic infections. For bovine babesiosis, we used *B. bovis* or *B. bigemina* merozoites concentrated by selective lysis of uninfected erythrocytes with 0.06M NaCl and solubilized by ultrasonication or 1% NP-40. Antigen concentration was 20 ng protein/dot. Two hundred bovine serum samples were analyzed using the DOT-ELISA and the indirect fluorescent antibody test (IFAT). Results were: *B. bovis*: sensitivity=90%, specificity=76%, predictive value=87%, *B. bigemina*: sensitivity=96%, specificity=86%, predictive value=96%. Agreement between DOT-ELISA and IFAT was 87% and 96%, for *B. bovis* and *B. bigemina*, respectively. Isolated, intact, *A. marginale* initial bodies were used at a concentration of 25 ng protein/dot. Antigen-antibody complexes were detected with AP-conjugated protein A. Overall performance of the DOT-ELISA with 580 bovine serum samples was: sensitivity=93%, specificity=96%, predictive value=95%. Agreement with IFAT was 93%. The simplicity and cost-effectiveness of the DOT-ELISA combined with high sensitivity and specificity indicate that this assay could effectively supplement or replace serologic assays currently used for bovine anaplasmosis and babesiosis.

K: TROPICAL VETERINARY MEDICINE

- 230 SURVEILLANCE OF HUMAN EHRLICHIOSIS IN THE UNITED STATES, 1988. Thomas R. Eng *, D.B. Fishbein, J.E. Dawson, N. Greene, M. Redus. Viral and Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA.

Human ehrlichiosis is an acute febrile illness caused by Ehrlichia canis or another closely related rickettsia. In 1988, we tested 210 serum pairs submitted for ehrlichiosis serology and 193 sera from patients who were suspected of having Rocky Mountain spotted fever. Patients residing in 35 states were tested. Thirty two patients had a four-fold or greater rise or fall in antibody titers to E. canis. An additional 10 patients had high but stationary titers. Excluding patients for whom the information was not available, 90% of confirmed patients were male and the median age was 44 years (range 4 to 77). Eighty-two percent of patients became ill between May and July. Patients resided in or were exposed to ticks in 11 states including 4 states where ehrlichiosis had not been reported previously. Eighty-four percent of patients recalled exposure to ticks in the 3-week period before their onset of illness. Of those bitten by ticks, the median incubation period was 9 days (range 4 - 33). Ninety-one percent of patients were hospitalized and 6% died. The most common initial symptoms and signs were fever, chills, headache, myalgia, and malaise. Ninety-five percent of patients had elevated levels of hepatic aminotransferases, and all had thrombocytopenia, leukopenia, or both.

- 231 IMMUNOGENICITY OF A SYNTHETIC PEPTIDE DERIVED FROM THE BabR LOCUS OF BABESIA BOVIS.

*G.L. McLaughlin, M.A. James, ¹M. Toro, and S. Montenegro-James. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL. and Instituto de Investigaciones Veterinarias Agropecuarias, Venezuela.

Attenuated Australian vaccine strains of Babesia bovis display genomic rearrangements at the C-terminal region of the BabR locus (Cowman et al, 1984, Cell 37: 653-660). By analyzing the open reading frame of the conserved N-terminal region of the BabR locus, a hydrophilic 10-amino acid peptide (DDESEFDKEK) was selected and synthesized. This peptide was coupled to hemocyanin using glutaraldehyde and injected into rabbits (1 mg) and cattle (4.5 mg) using Freund's adjuvant in a regime of 3 s/c injections every 2 weeks. Antibody responses to B. bovis were observed by indirect immunofluorescence (parasitic fluorescence) and by Western blot (3-4 specific bands, M.W. 90, 50, 20 KD) assays. Two weeks after the third immunization, 4 vaccinated and 4 control cattle were each challenged with 1.8×10^8 virulent Venezuelan B. bovis organisms. Differences in prepatent period, days of fever, packed cell volume, and weight loss were minimal; but significant decreases in maximum % parasitemias ($0.1 \pm 0.05\%$ vaccinates; $0.74 \pm 0.6\%$ controls) were observed. These results suggest that Latin American strains of Babesia contain this BabR epitope, and that additional study of this and other Babesia peptides may be warranted. Supported in part by Fondo Nacional de Investigaciones Agropecuarias (FONAIAP), Venezuela.

L: ENTOMOLOGY - SANDFLIES

- CHINKS IN THE BEHAVIORAL ARMATURE OF PHLEBOTOMUS PAPATASI
(DIPTERA: PSYCHODIDAE), THE VECTOR OF LEISHMANIASIS IN ISRAEL.
232 B. Yuval, A. Warburg and Y. Schlein. Hebrew University Medical School, Jerusalem, Israel.

Phlebotomus papatasi (Diptera: Psychodidae) is the sand fly vector of cutaneous leishmaniasis in the Jordan Valley, where it is associated with the sand rat Psammomys obesus (Rodentia: Gerbillidae), the main zoonotic reservoir of this disease. In order to identify a suitable target population for vector control by means of behavioral manipulation, physiological correlates to activity patterns of the sand fly around the rodents burrow were sought.

Proportions of males and females captured from dusk to midnight and from midnight to dawn were recorded, as were the following physiological distinctions: sugar feeding status of females and males; insemination, stage of ovarian development, time elapsed from last blood meal and parity in the females. The same comparisons were made between the total active population and dispersing flies. By comparing the sugar contents of flies leaving and entering the burrows, it was established that the main activity of gravid sand flies outside burrows is sugar feeding. Females, particularly parous ones, tended to disperse significantly more than males, yet blood fed females at advanced stages of the gonotrophic cycle were active almost exclusively in the first half of the night and did not disperse. Thus, this subset of the population blood fed, gravid, sugar seeking females- presents itself as a target for control by placing an attractive bait, containing sugar and one of a variety of biological control agents, in the vicinity of burrow openings.

- ON THE RECOGNITION MECHANISM BETWEEN LEISHMANIA FLAGELLA AND SAND-FLY MIDGUT EPITHELIUM. A.Warburg*, R.B.Tesh and D.McMahon-Pratt.
233 Department of Epidemiology and Public Health, Yale School of Medicine, P.O.Box 3333, New Haven, CT 06510. USA.

The ability of Leishmania promastigotes to attach to the midgut epithelium of their sand-fly hosts, constitutes an important stage in the establishment of the infection, enabling their subsequent transmission by bite. We developed an in vitro assay to study the recognition mechanism involved in this association. Frozen sections of sand-fly guts were incubated with Leishmania-flagella preparations, and probed with a flagella-specific monoclonal antibody. Tissue-specific adhesion of flagella to midgut epithelium was demonstrated by indirect immunofluorescence. None of the 13 sugars, screened for possible lectin-mediation, appeared to significantly inhibit the adhesion of flagella to midgut sections. Similarly no inhibition was achieved by incubating flagella with pep 63, an inhibitor of the promastigote-macrophage recognition mechanism. Significant inhibition was attained by incubating flagella preparations with a monoclonal antibody which binds to a component of the flagellar membrane.

The findings suggest that recognition of microvillar epithelium by the Leishmania flagellum, is biochemically mediated, apparently distinct from the recognition of the cuticular surfaces of the foregut and the hindgut, which is most likely affected by the physical characteristics of these surfaces. Adhesion of Leishmania flagella to sand-fly midgut sections was not confined to natural vector-parasite combinations. The described mechanism therefore, can not account for the conservativeness of these associations in nature.

L: ENTOMOLOGY - SANDFLIES

- 234 RECOVERY OF BARTONELLA BACILLIFORMIS FROM CRYOPRESERVED LUTZOMYIA VERRUCARUM AND L. PERUENSIS (Diptera: Psychodidae).

C.R. Latorre*, E. Rogers, S.E. Romero, and R. Fernandez.
U.S. Naval Medical Research Institute Detachment, Lima, Peru.

This study was undertaken to determine whether Lutzomyia species harbor B. bacilliformis, as reported by Noguchi, et al. (1929), and to test the survival of B. bacilliformis in sand flies preserved in liquid nitrogen. Female Lutzomyia were captured in a farm house in the Santa Eulalia Valley, Lima Department, Peru, and cryopreserved 78 to 174 days according to methods outlined by Morales et al. (1981). Flies were thawed, identified by examination of spermathecae, triturated in Schneider's medium supplemented with 15 % fetal bovine serum and passed through a 0.45 micron filter. Filtrates inoculated onto blood agar slants yielded typical colonies of Gram negative bacteria identified as B. bacilliformis by immunofluorescent tests, growth in red blood cells (Latorre et al., 1988), and morphology in Giemsa-stained thick and thin blood smears. A total of 24 individual specimens of L. verrucarum and 8 samples of pooled (N = 10) L. verrucarum were cultured: 13 individual cultures and 3 pools were contaminated; 8 individual cultures and 2 pools were negative; and 3 individual cultures and 4 pools yielded B. bacilliformis. Both cultures from 2 specimens of L. peruensis yielded B. bacilliformis. We conclude that B. bacilliformis survives cryopreservation and that the Noguchi et al. (1929) conclusions are correct regarding L. verrucarum. In addition, the presence of B. bacilliformis in field-collected L. peruensis, not previously substantiated, deserves further attention from epidemiologists.

- 235 SAND FLIES ASSOCIATED WITH HUMAN POPULATIONS IN THE REPUBLIC OF DJIBOUTI. *D.J. Fryauff, H.A. Hanafi, C. Bailly. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt; Service d'Hygiene, Ministre de Santé et Affaires Sociale, Republique de Djibouti.

Following the recent appearance of human cases of visceral leishmaniasis (VL) in the Republic of Djibouti, a series of country-wide entomological surveys were conducted to determine the presence of potential vectors of Leishmania and their associated ecology. Sand fly collections were restricted to 8 human population centers along an altitudinal transect extending from sea level to 1589 meters. Habitats ranged from open desert to mountain forest. Collections were done by means of castor oil paper traps and CDC light traps equipped with green chemical lights. Over 3,000 sand flies were collected, and of the 16 species identified, 6 (Phlebotomus orientalis, P. sergenti, Sergentomyia africanus, S. palestinensis, S. bedfordi, S. taizi) represent new geographic records for the country. Phlebotomus orientalis, a proven vector of VL, was collected repeatedly from only one type of habitat, the mountain forest, suggesting that its role in the transmission of VL may be ecologically restricted. Phlebotomus sergenti, a proven vector of CL, was collected in low numbers from only 2 highland villages. Two other anthropophilic species, P. bergeroti and P. alexandri were widely distributed over the country and frequently collected in large numbers. A laboratory colony of P. bergeroti has been established and efforts are currently being made to determine its vector competency for CL and Phlebotomus fever viruses. (Supported by NMRDC Work Unit No. 3M161102BS13.AD.310).

L: ENTOMOLOGY - SANDFLIES

236 BIOCHEMICAL TAXONOMY, ENZYME POLYMORPHISM, AND POPULATION GENETICS OF EGYPTIAN SAND FLY VECTORS OF LEISHMANIA.

*H.A. Kassem, Fryauff D.J., Shehata M.G., El Sawaf B.M. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt; Training Center for Research on Vectors of Diseases, Ain Shams University, Cairo, Egypt.

We have recently employed cellulose acetate enzyme electrophoresis (CAE) to distinguish between Phlebotomus papatasi and P. langeroni. These two anthropophilic sand fly species exist sympatrically at a focus of visceral leishmaniasis in Egypt. Our objective was to develop a non-morphological procedure for their identification. Working with both lab-reared and field-collected populations, 4 enzymes were found to be useful for electrophoretic characterization of the species. Epidemiological assessments of their populations and vector potentials can, as a result, be more accurately and efficiently undertaken. Procedures developed for separation and detection of sand fly enzymes are currently being applied to other problematic vector species, and the data generated from these studies is being used to determine genetic relationships among populations and species. Wild populations of P. papatasi from 3 locations in Egypt have been studied in this manner. While patterns of cutaneous leishmaniasis transmission appear to differ considerably among the three locations, results obtained from the analysis of 15 enzyme loci indicate no significant differences in the genetic composition of the three vector populations. (Supported by NMRDC Work Unit No. 3M161102BS13.AD.310 and NIH-NIAID Contract No. 01 AI22667/NIH-NIAID).

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POPULATION STUDIES OF PHLEBOTOMINE SAND FLIES IN THE BARINGO DISTRICT OF KENYA. *P.V. PERKINS,

P.G. LAWYER, G. KILU, Y. MEBRAHTU, J. NZOVU,

J.I. GITHURE, AND I.O. OUMA. Kenya Medical Research

Institute, Nairobi, Kenya. US Army Medical Research Unit, Nairobi, Kenya. Walter Reed Army Institute of Research, Washington, DC.

A continuous four year sand fly survey was conducted in an endemic cutaneous and visceral leishmaniasis area of Baringo District, Rift Valley Province, Kenya. Daily collections using light traps, sticky paper traps, and aspirators were made. Efforts concentrated on collecting sand flies in or near homes where human cases of kala-azar Leishmania donovani had occurred. Over the four years more than 80,000 sand flies were collected and identified to species. The dissection of over 30,000 female sand flies provided parasite cultures and isolates identified as Leishmania donovani, Leishmania major, and Leishmania adleri in addition to unknown cultures of flagellated Leishmania-like protozoans. The sand fly resting habitats sampled included the huts of kala-azar patients and nearby termite hills, animal burrows, tree holes and rock crevices. Four Phlebotomus and ten Sergentomyia species were routinely trapped or collected. This field-based, laboratory-supported study of the seasonal population fluctuations and infection rates during rainy and dry seasons has provided insight into transmission of kala-azar in this part of Kenya.

ASTMH PRESIDENTIAL ADDRESS
(No abstract available)

- 238 BIOLOGY OF MALARIA. L.H. Miller. Malaria Section, Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

ASTMH ANNUAL BUSINESS MEETING

ASTMH POSTER SESSION II

- 239 RELEASE OF MEROZOITE DENSE GRANULES DURING ERYTHROCYTE INVASION BY PLASMODIUM KNOWLESI

*M. Torii, J.H. Adams, L.H. Miller and M. Aikawa. Case Western Reserve University and National Institute of Health, Bethesda, Maryland

We studied the fate of dense granule contents during erythrocyte entry by the merozoite with immunoelectron microscopy using antibodies which react specifically with P. knowlesi merozoite dense granules. Once merozoites entered host cells, dense granules moved to the pellicle and released their contents into the parasitophorous vacuole space. The dense granule material did not disperse in the parasitophorous vacuole but remained associated with the merozoite surface as a granular mass. The dense granule material then entered finger-like channels of the parasitophorous vacuole which projected into the erythrocyte cytoplasm. This is the first report showing that the contents of dense granules of Plasmodium are different from rhoptries and micronemes and associated with the formation of channels from the parasitophorous vacuole.

ASTMH POSTER SESSION II

240 OCTAPEPTIDE EPITOPES IN PLASMODIUM FALCIPARUM CAMP STRAIN MEROZOITE SURFACE ANTIGEN DETECTED WITH AOTUS ANTIBODIES.

J. M. Carter, A. W. Thomas, and J. A. Lyon. Walter Reed Army Institute of Research, Div. CD&I, Department of Immunology, Washington, DC 20307-5100.

The precursor to the major surface protein of Plasmodium falciparum merozoites is a 195 kDa glycoprotein (gp195). Immune responses to gp195 can control blood stage disease [review: A.A. Holder, in Progress in Allergy, 41:72-97, (Karger, Basel) 1988]. This makes the protein an attractive candidate for a malaria vaccine. In this study, we show that antibodies in protective immune serum recognize several continuous (sequential) epitopes throughout gp195. These results were obtained by using ELISA to detect reaction between serum antibody and solid phase overlapping octapeptides [H.M. Geysen, et al, J. Immunological Methods, 102:259-274, 1987] from the CAMP strain protein. In order to determine if these continuous epitopes play a role in malarial immunity, antibodies dissociated from immune complexes present in immune clusters of merozoites (ICM) [J.A. Lyon, et al, J. Immunol., 136:2252-2258, 1986] were also evaluated. Little of the antibody that dissociated from the immune complexes reacted with the peptides whereas similar amounts of these ICM antibodies easily detected processed products of gp195 and fragments expressed through recombinant methods. These results suggest that most of the antibodies that inhibit merozoite dispersal react with discontinuous (conformational) epitopes.

241 COMPARISON OF INVASION EFFICIENCIES OF ERYTHROCYTES FROM DIFFERENT MOUSE STRAINS BY PLASMODIUM FALCIPARUM MEROZOITES. Francis W. Klotz*, Palmer Orlandi, J. David Haynes, Stuart J. Cohen, Gert Reuter, Roland Schauer, Russell J. Howard and Louis H. Miller. Walter Reed Army Institute of Research, Washington, D.C., Biochemisches Institut, Christian-Albrecht's Universität, Kiel, West Germany, DNAX, Palo Alto, C.A. and the National Institutes of Health, Bethesda, M.D.

Plasmodium falciparum merozoites invade murine erythrocytes in vitro. To characterize erythrocyte determinants on mouse cells required for invasion, we compared the invasion efficiencies of three clones of P. falciparum into erythrocytes of different mouse strains. Comparison of invasion between different mouse cells and human controls indicated that DBA/2 mouse cells were invaded more efficiently than AKR or C₅₇BL/6:

Mouse Strain	Parasite		
	Malayan Camp	Brazilian 7G8	Thai TN
AKR	20%	19%	14%
C ₅₇ BL/6	22%	14%	9%
DBA/2	41%	42%	38%

Binding of the 175 kDa P. falciparum sialic acid binding receptor (EBA-175) to mouse cells was also compared. EBA-175 bound DBA/2 cells more efficiently than AKR or C₅₇BL/6. These data suggest that invasion of mouse cells is dependent upon erythrocyte sialic acids. Mouse cells contain sialic acid as N-acetyl neuraminic acid and also its N-glycolyl and 9-O-acetyl forms. These alternative forms of sialic acid may compete with the natural ligand to reduce EBA-175 binding and invasion. Such naturally occurring sialic acid analogs may provide insight into the sialic acid-dependent interactions during erythrocyte invasion by P. falciparum.

ASTMH POSTER SESSION II

- 242 ERYTHROPOIESIS DURING LETHAL AND NONLETHAL PLASMODIUM YOELII INFECTIONS IN MICE. K.L. Miller*, P.H. Silverman and B. Kullgren. Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA.

The anemia of malaria is complex and contributes to the mortality and morbidity of the disease. Both an increased destruction and a decreased production of red blood cells is involved, however, the mechanisms remain unclear. We have shown that tumor necrosis factor alpha (TNF), produced by macrophages in response to infection, contributes to the anemia of malaria through its ability to inhibit erythropoiesis. The ability to compensate the anemia of malaria has been suggested to play a role in resistance to infection. Differences in the production of TNF during lethal and nonlethal infections may effect the ability of mice to expand erythropoiesis in response to the anemia. We therefore examined erythropoiesis and TNF production in mice during infection with lethal and nonlethal strains of Plasmodium yoelii. Erythroid progenitor cells, colony forming unit-erythroid (CFU-E) and burst forming unit-erythroid (BFU-E), were present in reduced numbers in the bone marrow of mice infected with both strains of P. yoelii. The reduction of erythroid progenitor cells was more significant in mice infected with the lethal strain. For example: on day 2 postinfection, BFU-E/femur were 78% of control in mice infected with the nonlethal strain and 54% of control in mice infected with the lethal strain. In addition, mice infected with the lethal strain showed reduced ability to compensate the anemia by expanding CFU-E. These results suggest that alterations in erythropoiesis may contribute to the severity of infection. The production of TNF during the course of both lethal and nonlethal infections is currently being investigated.

- 243 DEFINED PLASMA-FREE MEDIA FOR THE CULTIVATION OF PLASMODIUM FALCIPARUM. V.C. Okoye and *S.K. Martin. Walter Reed Army Institute of Research, Washington, DC 20307-5100

The obligatory requirement for plasma in the continuous cultivation of P. falciparum limits the application of this valuable technique. Plasma is undefined, its supply erratic and lot to lot variation sometimes leads to inconsistent results. We have identified a commercially available plasma-free medium (AIM V) and defined a simpler alternate (BAM) that supports the growth and multiplication of several P. falciparum strains. Multiplication rates of the Honduran strain HB-3 in the two plasma-free media were compared to that in human plasma supplemented medium (HPM). AIM-V was used directly in culture without modification. HPM and BAM were 10% human type A plasma and 0.5% fatty acid free bovine albumin solutions, respectively in RPMI-1640 to which Hepes and NaHCO₃ had been added. Starting parasitemia was 0.05% in each medium and parasites were subcultured every 5th day with type O human red cells to a parasitemia of 0.2%. Table shows parasitemia on day of subculture.

Day	0	5	10	15	20	25
AIM-V	0.05	2.8	4.5	4.6	4.5	5.4
BAM	0.05	2.9	3.0	3.0	4.0	4.1
HPM	0.05	4.3	5.1	7.4	4.9	6.9

P. falciparum malaria parasites can be successfully cultivated in defined plasma-free media.

ASTMH POSTER SESSION II

- 244 A NON-SIALIC ACID DEPENDENT PATHWAY OF INVASION CAN BE INDUCED IN A Plasmodium falciparum CLONE BY CULTIVATION IN NEURAMINADASE TREATED ERYTHROCYTES
 *Stephen A. Dolan, Louis H. Miller, Thomas E. Wellems. Malaria Section, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

We wondered whether Plasmodium falciparum clones which invade erythrocytes principally via sialic acid dependent pathways could develop alternative invasion pathways. We studied six clones of Plasmodium falciparum and characterized their ability to proliferate in culture (percent parasitized erythrocytes) and to invade normal and neuraminadase treated erythrocytes. Three clones were sialic acid dependent with invasion rates of neuraminadase treated erythrocytes of 10-20 % of control and inability to proliferate in neuraminadase treated erythrocytes. Three clones were able to invade neuraminadase treated erythrocytes at rates approximating 50 % of control and to proliferate, albeit at reduced rates, in neuraminadase treated erythrocytes. To determine whether a sialic acid dependent clone was capable of adaptation to growth in neuraminadase treated erythrocytes clone Dd2 was propagated in neuraminadase treated erythrocytes. After several days in culture a population of parasites, Dd2/NM, developed which was capable of invasion and proliferation in neuraminadase treated erythrocytes at rates approximating that of Dd2 in normal erythrocytes. This ability persisted after Dd2/NM was returned to normal erythrocytes for several growth cycles. Five subclones of Dd2 were obtained and behaved similarly. Pulsed field chromosomal analysis, DNA hybridization studies and erythrocyte binding assays demonstrate no differences between Dd2 and Dd2/NM. In conclusion, Plasmodium falciparum clones invade erythrocytes via sialic acid dependent and sialic acid independent pathways. Sialic acid dependence in clone Dd2 was overcome and the sialic acid independent character was stable over time in the absence of selection pressure.

- 245 HUMAN IMMUNE RESPONSE TO MALARIAL HEAT SHOCK PROTEINS

Ying Zhao*, Patricia Graves and Nirbhay Kumar. Imm Inf. Dis., SHPH Johns Hopkins University, Baltimore, MD and Q.I.M.R., Brisbane, Australia.

Stress proteins present in all eukaryotes and prokaryotes have been shown to be immune targets in a broad range of infections. We have analyzed sera from people living in Papua New Guinea after exposure to Plasmodium falciparum for specific antibodies against two heat shock related proteins (proteins similar to the heat shock protein of m.w. 70,000, Pfhs and a glucose regulated protein of m.w. 78,000, Pfgr). Using immunoprecipitation analysis and synthetic peptides in ELISA, specific antibodies against Pfhs and Pfgr were detected in the sera of these infected individuals. Sera from people exposed to a different human malaria parasite P. vivax, did not react with the peptides in ELISA. Southern blot analysis with DNA isolated from P. falciparum of different geographical location showed conservation of genes for these stress proteins and thus these proteins are equally likely to be immune targets in various endemic areas. Lymphocytes from two immune donors tested, responded in proliferation assays to purified Pfhs/Pfgr and purified recombinant proteins. However, a similar response was also seen in lymphocytes from non-immune individuals and thus has raised questions pertaining to a generalized responsiveness of lymphocytes to some common determinants present in these groups of proteins in various pathogens.

- 246 **CHARACTERIZATION OF ANTIBODY RESPONSES INDUCED BY DIFFERENT SYNTHETIC ADJUVANTS TO THE *P. falciparum* MAJOR MEROZOITE SURFACE PRECURSOR PROTEIN, GP195.** G.S.N.Hui⁺, S.P.Chang⁺, L.O.Tam⁺, A.Kato⁺, S.E.Case⁺⁺, C.Hashiro⁺, S.Kotani⁺⁺, T.Shiba⁺⁺, S.Kusumoto⁺⁺, and W.A.Siddiqui⁺. ⁺ Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu, Hawaii. ⁺⁺ Osaka College of Medical Technology, Osaka, Japan.

A successful vaccine against malaria may require considerable efforts in the development of a safe and effective adjuvant formulation. We have found that rabbits immunized with the *P. falciparum* merozoite surface precursor protein (gp195) using a combination of two synthetic immunomodulators, B30-MDP and LA-15-PH (MPL equivalent) in liposomes produced antibody titers equal to or greater than FCA. Moreover, these antibodies were able to inhibit parasite growth *in vitro*. In order to study the role of these synthetic adjuvants in gp195-specific immune responses, mice (Swiss Webster and C57Bl/10 SnJ) were immunized with purified gp195 using FCA, Alum (Alhydrogel), B30-MDP/liposome, LA-15-PH/liposome, B30-MDP/LA-15-PH/liposome, or liposome alone as adjuvants. In C57Bl mice, LA-15-PH or in combination with B30-MDP produced the higher antibody responses, as measured by an ELISA assay, than FCA or B30-MDP alone. Differences in responses were not apparent in outbred Swiss Webster strain immunized with most adjuvant formulations. In both mouse strains, alum induced little or no primary responses as detected by ELISA, and after three injections only very low levels of gp195-specific antibodies were produced. The ability of these adjuvants to elicit gp195-specific antibodies of different isotypes were also examined. All adjuvants were able to induce IgG1 and IgM production. LA-15-PH enhanced the production of gp195-specific IgG2b (in C57Bl mice) and, IgG2a and IgG2b (in Swiss mice) over that of B30-MDP. In both mouse strains, very low levels of antigen specific IgG3 were detected when gp195 was emulsified in FCA. However, incorporation of the antigen in multilamellar liposomes alone can significantly enhance the production of this isotype. None of the adjuvants studied were able to induce gp195-specific IgG2a in C57Bl mice. These results suggest that gp195 incorporated in LA-15-PH/liposome alone or with the MDP derivative can elicit higher humoral response and/or broader isotype distribution than when given with FCA or B30-MDP. The specificity of antibodies induced by these immunomodulators for different B-cell epitopes of gp195 are currently being investigated. (Supported by USAID)

- 247 **GROWTH INHIBITORY ANTIBODIES INDUCED IN RABBITS USING THE COMBINATION OF SYNTHETIC ADJUVANTS B30-MDP AND LA-15-PH WITH GP195, A PROMINENT MALARIA VACCINE CANDIDATE** L.O. Tam⁺⁺, G.S.N. Hui⁺, J. Kagemoto⁺, S. Kotani⁺⁺, T. Shiba⁺⁺, S. Kusumoto⁺⁺, and W.A. Siddiqui⁺. ⁺Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu and ⁺⁺Osaka College of Medical Technology, Osaka, Japan

We have shown that "native" gp195 can completely protect *Aotus* monkeys against a lethal challenge of virulent, homologous *P. falciparum* FUP parasites; however, three injections of Freund's complete adjuvant (FCA) were required. Since FCA is unacceptable for clinical use, we have sought to establish a synthetic, low toxicity adjuvant formulation which can effectively replace FCA. B30-MDP and LA-15-PH are two low toxicity, fully synthetic immunomodulators which are undergoing clinical development. B30-MDP is an MDP derivative shown to potentiate cell-mediated immune responses, while LA-15-PH is the synthetic equivalent of the Lipid A derivative monophosphoryl lipid A, recently shown to augment humoral immune responses by abrogation of suppressor T cell activity. We have immunized rabbits with native gp195 presented with the combination of B30-MDP and LA-15-PH in multilamellar liposomes and found that antibody titers were equivalent to or greater than the titers achieved in rabbits given gp195 three times in FCA. More importantly, serum samples from rabbits inhibited parasite growth *in vitro* by 71%. In a previous study, we found that serum samples from *Aotus* monkeys immunized with gp195 three times in FCA and having a mean peak parasitemia, following lethal challenge, of 2.3% (A368, A372 and A375) gave a mean inhibition of 57% (Hui, G. and W.A. Siddiqui, *Exp. Parasitol.* 64:519-522, 1987). These data indicate that this combination of synthetic immunomodulators may be capable of replacing FCA in monkey vaccination experiments designed to evaluate the protective activity of native gp195 processing fragments and gp195-based recombinant polypeptides. (Supported by USAID).

ASTMH POSTER SESSION II

248 A NOVEL 230kDa PLASMODIUM FALCIPARUM ANTIGEN MAY BE A TARGET OF IMMUNE ATTACK.

I.N. Ploton, A.W. Thomas, J.M. Carter and J.A. Lyon. Dept. Immunology, Walter Reed Army Institute of Research, Washington DC 20307-5100.

Antigens involved in the formation of immune clusters of merozoites (ICM'S), when blood-stage schizont-infected erythrocytes are matured in the presence of immune serum, are attractive vaccine candidates (1). We have isolated a clone, expressing antigenic determinants of a 230kDa ICM antigen, from a P. falciparum CAMP strain genomic DNA lambda gt11 library. The deduced sequence of this insert is in frame with beta-galactosidase. Overlapping restriction enzyme fragments have also been cloned and sequenced. The open reading frame contains at least two different repeat regions. One of these regions includes a Glu-Glu dipeptide, as has been reported for some other malarial proteins (2). Comparison of the nucleotide and deduced amino-acid sequence of this antigen with various sequence databases shows no overall homology with any other reported sequences. Peptides corresponding to repeat and non-repeat regions have been synthesized and data utilizing these peptides to further characterize the 230 kDa antigen will be presented.

1. Lyon J.A., Thomas A.W., Hall T. and Chulay J.D. Mol. Biochem. Parasitol. (1989) in press.
2. Mattei D., Berzins K., Wahlgren M. et al. Parasit. Immunol. (1989) 11 : 15-30

249 IDENTIFICATION AND CHARACTERIZATION OF THE 50kDa ANTIGEN INVOLVED IN PLASMODIUM FALCIPARUM ICM FORMATION.

D.A. Carr, J.A. Lyon, and A.W. Thomas. Dept. Immunology, Walter Reed Army Institute of Research, Washington DC 20307-5100

A subset of P. falciparum antigens are involved in the formation of immune clusters of merozoites (ICM's) when blood-stage parasites mature in the presence of immune serum (1). We will present the sequence of a lambda gt11 CAMP strain genomic clone that affinity selects antibody specific for a 50kDa antigen involved in ICM formation. The sequence has homology with MSA II, a merozoite surface glycoprotein whose sequence has been reported for the FCQ27 strain (2). These sequences share very similar N and C terminal regions flanking a highly variable region of approximately 140 amino-acids. Computer models indicate that secondary structure is not conserved in this variable region. Disparity between deduced and apparent M.W's of CAMP MSA II suggest that glycosylation is extensive. For vaccine development it is critical to establish whether the peptide, and in particular its conserved regions, are available to immune attack on intact merozoites. We will present evidence addressing these questions.

1. Lyon JA., Thomas AW., Hall T. and Chulay JD. Mol. Biochem. Parasitol. (1989) in press
2. Smythe JA., Coppel RL., Brown GV., et al PNAS (1988) 85:5195

ASTMH POSTER SESSION II

250 SEQUENCE DIVERGENCE BETWEEN THE ERYTHROCYTE BINDING
ANTIGENS (EBA-175) OF THE CAMP AND FCR-3 STRAINS OF
PLASMODIUM FALCIPARUM.

*Lisa A. Medvitz and David E. Lanar. Walter Reed Army Institute of Research, Washington D.C.

A 175 kDa Plasmodium falciparum blood stage antigen which binds to erythrocytes and merozoites, designated EBA-175, appears to be involved in the initial attachment of the merozoite to the erythrocyte. Previous analysis of EBA-175 from the CAMP and FCR-3 strains of P. falciparum showed that while there was no difference in the binding of EBA-175 to the susceptible erythrocytes, there was strain specificity evident when the EBA binds to the merozoite (Camus & Hadley, Science, 1985; 230: 553). In order to investigate this difference in binding at the molecular level, we have cloned and sequenced a 3.4 kilobase gene from the FCR-3 strain of P. falciparum and compared it to the sequence of the CAMP strain EBA-175 (Sim et.al., manuscript submitted). While there is virtual sequence homology between the two strains, there are significant areas of complete divergence. The CAMP strain sequence contains a 342 bp segment not present in the FCR-3 strain and the FCR-3 strain sequence contains 423 bp segment not present in the CAMP strain. Deduced polypeptides of both regions have low pI values of about 3.8. These areas of divergence may be of significance in the differential binding to merozoites observed between the two EBA-175 proteins of the CAMP and FCR-3 strains.

251 Individuals Produce Different Repertoires of Antibodies that Inhibit
Malaria Merozoite Dispersal. *J. A. Lyon and A. W. Thomas, Department
of Immunology, Walter Reed Army Institute of Research, Washington D.C.

Immune Clusters of Merozoites (ICM) form when antibodies in protective sera inhibit merozoite dispersal from erythrocytes infected with Plasmodium falciparum malaria parasites. When ICM were prepared with serum from Aotus monkey A076 and extracted with isotonic buffer, pH 8.0, containing 1% Triton X-100 (TX-100), immune complexes containing antigens corresponding to a 230/215 kDa doublet, the major merozoite surface antigen (gp195), the Serine Repeat Antigen (SERA), the glycophorin binding protein (GBP130), a 150 kDa and a 127 kDa protein partitioned preferentially to the TX-100 insoluble fraction. Immune complexes containing Acidic Basic Repeat Antigen (ABRA), Merozoite Surface Antigen 2 (MSA 2), and a 30 kDa protein partitioned preferentially to the TX-100 soluble fraction.¹ Most likely, immune complexes in the TX-100 insoluble fraction are cross-linked by antibodies but those in the TX-100 soluble fraction are not because antigen density or epitope accessibility is limited or antibody response is limited. Analyzing immune complexes from ICM prepared with additional monkey sera (A029 and A072) and a human serum (VDB) gave different results. Immune complexes containing MSA 2 were found preferentially in the TX-100 insoluble fraction of ICM prepared with A029 antibodies; no immune complexes containing SERA were obtained from ICM made with A072 antibodies; and significantly larger quantities of immune complexes containing GBP130 were obtained from ICM made with VDB serum than from the monkey sera. These results show that individuals produce different repertoires of antibodies that inhibit malaria merozoite dispersal and may influence the selection of antigens used in vaccine development.

¹ Lyon et al. (1989), Mol. Biochem. Parasitol. (in press)

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- 252 IDENTIFICATION OF A NOVEL 4 KB REPETITIVE UNIT FROM PLASMODIUM FALCIPARUM. J.R. Plitt, G.-X. Chen, A.K. Weiler, J. Zhu, and *J.W. Zolg. Biomedical Research Institute, Rockville, MD and University of Düsseldorf, Düsseldorf, FRG.

To improve detection assays based on 21 bp repetitive units specific for P. falciparum, we developed a capture assay integrating a novel type of repetitive P. falciparum DNA (PF-DNA). Genomic PF-DNA was digested with Dra I (AAATTT) and Southern blots were probed with PF-DNA, the 21 bp repeats, and P. berghei DNA. With PF-DNA as a probe, a series of bands was seen, ranging from 10 kb to 0.5 kb. In contrast, the 21 bp probe hybridized only in the high molecular weight range, predominantly recognizing fragments identical to those already visible in Dra I digests of PF-DNA after ethidium bromide staining of agarose gels. A 4 kb fragment was detected in blots probed with PF-DNA which was absent when the 21 bp repeats or P. berghei DNA were used as a probe. No cross-hybridization was seen between the 4 kb repeat and both human and P. vivax DNA.

Probing Dra I digests from 10 different P. falciparum isolates with the 4 kb fragment revealed a strain-specific arrangement of this repeat within the genome. The partial DNA sequence established so far seems not to be homologous to any of the different classes of repeats of plasmodial origin previously described. PCR amplification of parts of the 4 kb repeat revealed the presence of degenerated internal repeats. (Supported by Contract DPE-0453-A-00-4036-00 from U.S.A.I.D.).

- 253 BLOOD STAGE PLASMODIUM YOELII INFECTION DOES NOT STIMULATE PROTECTIVE CD8+ T CELL IMMUNE RESPONSES. Joseph M. Vinetz, Sanjai Kumar, Michael F. Good, Jay A. Berzofsky, and Louis H. Miller.

The malaria parasite, Plasmodium yoelii 17X causes a self-limited, non-lethal infection of mice characterized, in the blood stage, by preferential invasion of reticulocytes. Previous studies have suggested that immunity to the blood stage infection may be related to enhanced levels of class I MHC antigens on the parasitized reticulocyte surface and that immunity can be transferred to immunodeficient mice by immune CD8+ as well as CD4+ T cells. These phenomena would necessitate novel concepts of how processing and presentation of class I MHC-presented antigens occurs. To further examine the mechanisms of CD8+ T cell involvement in immunity to blood stage P. yoelii infection, our laboratory performed in vivo CD8 T cell depletion studies and adoptive transfer experiments. Depletion of CD8+ T cells during primary blood stage infection in BALB/c mice had no influence on the course of infection. Adoptive transfer of immune spleen cells to immunodeficient mice of three different strains also showed no role for CD8+ T cells in immunity. Spleen cells from 102 week immune BALB/c and C57BL10 mice were transferred to BALB/c-nu/nu and C57BL10-nu/nu mice, respectively. CD8+ T cells were transferred from 1-2 week immune CBA/CaJ donors to in vivo CD4+ T cell-depleted CBA/CaJ recipients. While immune unfractionated spleen cells and CD4+ T cells transferred rapid protection in all three mouse strains, CD8+ T cells from immune donors by themselves were neither protective nor did they enhance immunity. These in vivo findings have important implications for fundamental immunological mechanisms.

ASTMH POSTER SESSION II

- 254 EXPRESSION OF DOMAINS OF *PLASMODIUM FALCIPARUM* gp195 IN YEAST.
 *H.L. Gibson, I.C. Bathurst, P.J. Barr, G.S.N. Hui, S.P. Chang and W.A. Siddiqui. Chiron Corporation, Emeryville, CA and Department of Tropical Medicine, University of Hawaii, Honolulu, HI.

The major merozoite surface protein (gp195) of *Plasmodium falciparum* is a candidate vaccine against malaria. Our groups have reported previously on the cloning of the gp195 gene from the FUP strain of *P. falciparum*, and the expression of amino-terminal domains of gp195 in the yeast *Saccharomyces cerevisiae*. More recently, we have extended our expression studies to include further amino-terminal regions of gp195 and also, regions of the protein adjacent to the hydrophobic carboxy terminus. This region has been proposed to correspond to an *in vivo* processing fragment of gp195 designated p42. Constructions for the intracellular expression of p42 gave relatively low yields of recombinant protein. This polypeptide could, however, be purified to homogeneity by affinity chromatography. Higher yields of p42 polypeptides were obtained using a yeast secretion system. The purified recombinant proteins were evaluated for immunogenicity in animal models, and the ability of the resulting antisera to recognize natural gp195 and blood stage merozoites was assessed. In addition, the ability of antisera to inhibit parasite growth in an *in vitro* assay was measured. This latter assay is a good indicator of the potential efficacy of a particular immunogen/adjuvant formulation for challenge studies in primates.

- 255 IDENTIFICATION OF *PLASMODIUM CHABAUDI ADAMI* ANTIGENS WHICH ELICIT PROTECTIVE IMMUNITY IN MICE.
 *P.E. Kima and C.A. Long. Hahnemann University, Philadelphia, PA

There is ample evidence for the role of cell-mediated immunity in protection to the erythrocytic stages of malarial parasites. The rodent malarial parasite *Plasmodium chabaudi adami* (PCA) provides a model in which resolution of acute infection is T-cell dependent. T-cell lines and a T-cell clone have been described which adoptively protect nude mice against challenge infection with this parasite. We have begun to investigate the plasmodial antigens which can elicit protective immune responses in this model. As one approach to this problem, naive mice were immunized with lysates of PCA-parasitized erythrocytes or PCA antigens fractionated by SDS-PAGE. Unfractionated antigen preparations derived either from early or late stages of erythrocytic development gave significant protection when administered in complete Freund's adjuvant. Sera obtained from these two groups of mice showed different patterns of reactivity when used to immunoprecipitate metabolically labeled PCA antigen. These antigen preparations were then fractionated by SDS-PAGE, and pools representing different molecular weight fractions were tested for their capacity to immunize mice against a PCA challenge. Two different size classes of polypeptides (20 kd to 35 kd and 90 to 100 kd) provided some protection to mice. Sub-fractions from these pools are being tested to characterize the specific antigens responsible for protection. This approach should lead to identification of antigens which can elicit protective immune responses in this model system. (This work was supported by Public Health Service Grant AI24015 from the National Institute of Allergy and Infectious Diseases.)

ASTMH POSTER SESSION II

256 RECOMBINANT SERA ANTIGENS PRODUCED IN THE YEAST
SACCHAROMYCES CEREVISIAE.

*K.M. Green, H.L. Gibson, I.C. Bathurst, P.J. Barr, W.Li, D.J. Bzik
and J. Inselberg. Chiron Corporation, Emeryville, CA and Dartmouth
Medical School, Hanover, NH.

The serine-repeat antigen (SERA) is a 989 amino acid, secreted, blood stage antigen of Plasmodium falciparum. Monoclonal antibodies to SERA have been shown to be inhibitory towards parasite growth in vitro, and the antigen itself has been shown to stimulate protective immunity in Saimiri monkeys. The availability of large quantities of recombinant DNA-derived SERA antigens will allow a full assessment of the potential of SERA as a human malaria vaccine component. Accordingly, we have used the yeast Saccharomyces cerevisiae for the production of SERA and various sub-domains of SERA as intracellular products. In many cases, direct expression of SERA molecules gave low levels of recombinant products. We have, therefore, used fusion protein strategies for increased production. Most notably, we have taken advantage of a novel expression system that uses yeast ubiquitin as the fusion partner. This strategy not only allows for higher levels of expression, but also gives rise to products that are cleaved from ubiquitin in vivo, by an endogenous yeast hydrolase.

257 IMMUNOGENICITY AND PROTECTIVE EFFICACY OF PLASMODIUM FALCIPARUM
CULTURE-DERIVED EXOANTIGENS. I. Kakoma,* M. A. James, C.
Fajfar-Whetstone, P. Buese, R. Hansen, G. Clabaugh, and M. Ristic,
College of Veterinary Medicine, University of Illinois, 2001 South Lincoln,
Urbana, IL 61801

A major glycoprotein (83 kD) was purified to homogeneity by high performance liquid chromatography (HPLC). A synthetic peptide (29 mer) was constructed from the N-terminal amino acid sequence (residues 3-31) of the 83 kD polypeptide. The 29 mer-associated epitopes were demonstrated to be widely conserved among S.E. Asian, S. American, and African isolates. The 29 mer-specific antibodies inhibited Plasmodium falciparum growth in vitro. Polypeptides, purified with the anti-29 mer MABS, were used as "vaccine" in a trial using Aotus nancymai monkeys. The immunogen fortified with saponin and aluminum hydroxide primed the vaccinates in such a way that a strong response to a wide range of polypeptides (25, 34, 70, 77, 100, 120, and 200 kD) was observed in Western immunoblots. Protection in vaccinated animals, challenged with the Indochina I/CDC strain, was evidenced by significantly ($P < 0.05$) lower decreases in hematocrit and hemoglobin levels and a markedly less severe clinical course of disease after challenge.

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258 USE OF SYNTHETIC PEPTIDES IN THE DETECTION OF PLASMODIUM FALCIPARUM BLOOD-STATE ANTIBODIES IN RESIDENTS OF BOLIVAR STATE, VENEZUELA.

M.A. James*, S. Montenegro-James, O. Noya, and F. Riggione. Dept. of Tropical Medicine, Tulane Univ. Med. Ctr., New Orleans, LA, and Universidad Central de Venezuela, Instituto de Medicina Tropical, Caracas, Venezuela.

We have previously reported the strong antigenicity of 4 synthetic peptides (29mer=C3, C2, C5, C10) constructed from internal chymotryptic digests of a 70kd P. falciparum Indochina I exoantigen. The use of such peptides in specific and sensitive enzyme immunoassays with appropriate treatment regimes may be of vital importance in reducing transmission of P. falciparum, particularly in areas on the threshold between hyperendemic and mesoendemic malaria, e.g., Venezuela. Since 1984, the number of confirmed cases of malaria in Venezuela has continued to rise, especially in Bolivar State; by the end of 1988, 23.4% of the state residents had been infected with malaria, an increase of 227.7% from the preceding year. Of 166 sera from individuals known positive of P. falciparum (IFA test), anti-peptide ELISA reactivity was as follows: 29mer=75%, C2=60%, C10=31%, C5=23%. Anti-peptide antibodies were also characterized for isotype-specificity, and reactivity with native malarial polypeptides (derived from local Venezuelan strains) in Western blots. Correlations were made between anti-peptide activity and recognition of particular P. falciparum antigens as to primary vs. recurrent P. falciparum infections, age and immune status/clinical characteristics of the individual.

259 EPIDEMIOLOGY AND IMMUNOLOGY OF P. FALCIPARUM MALARIA IN A POPULATION CONTAINING IMMUNE AND NONIMMUNE ADULT SUBJECTS. *T.R. Jones, J.K. Baird, S. Ratiwayanto, H. Hadiputranto, B. Leksana, and H. Basri. U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta, Indonesia

We studied two populations of adult male subjects, natives of Irian Jaya who have had lifelong exposure to hyperendemic falciparum malaria (n=54) and Javanese transmigrants having experienced exposure for only 2.5 years (n=67). Both the natives of Irian Jaya and the Javanese transmigrants were given radical cures for malaria (quinine, doxycycline, primaquine). After treatment, the transmigrants acquired new cases of P. falciparum malaria at a significantly higher rate than the Irianese subjects ($p < 0.001$). Fifty percent of the transmigrants were positive by week 9.5 while 50% of the Irianese became positive at week 16. Sera taken from the subjects were tested for reactivity to ring-infected erythrocyte antigen (RESA), whole, fixed blood stage malaria parasites and a solubilized parasite antigen preparation. Irianese subjects had significantly higher IgG titers to whole, fixed parasites ($p < 0.05$) and higher IgM titers ($p < 0.025$) to the soluble antigen preparation. They also had, as a group, a higher mean spleen size ($p < 0.001$). While several of the humoral immune parameters correlated well with each other, accurate predictors of susceptibility to malaria were not found. The transmigrants showed clear evidence of a humoral response to falciparum malaria. This response developed in only 2.5 years. We know this because Javanese who did not transmigrate have immune profiles similar to completely naive American volunteers and dissimilar to those noted in transmigrants. The immune response generated by the transmigrants was not, however, sufficient to provide protection as great as that seen in subjects who had spent their lives in a hyperendemic malarious environment.

ASTMH POSTER SESSION II

260 AGE-DEPENDENT, CHRONIC EXPOSURE-INDEPENDENT HUMORAL IMMUNE RESPONSES TO HYPERENDEMIC FALCIPARUM MALARIA IN ARSO PIR, IRIAN JAYA.

*J.K. Baird, T.R. Jones, M.J. Bangs, S. Tirtokusumo, and I. Wiady.
U.S.Naval Medical Research Unit No. 2, Jakarta Detachment, APO San Francisco 96356-5000

Increasing immune protection with age of lifelong residents of hyper- or holoendemic malarious areas is widely considered a product of chronic heavy exposure. We studied humoral immune responses in people of all ages > 2 years who had lived in an area of hyperendemic malaria for only 21 months. They had come from central and east Java where malaria transmission is very low compared to their new home in Irian Jaya (annual slide positive rate < 2% vs. > 40%, spleen rate < 1% vs. 60 to 95%). Regression analyses of age vs. IgG immunoassays for RESA, whole asexual blood stage parasite antigen, whole gametocyte parasite antigen, and solubilized asexual parasite antigen, all showed significant positive regression ($p < 0.001$ to $p < 0.05$). This was also true of lifelong residents of Irian Jaya who lived with the people from Java, and the slopes of matched regression lines for the two subpopulations were equal ($p > 0.10$ to $p > 0.75$) for each immunoassay. In contrast, people who still lived in Java showed IgG levels for all the antigens which were comparable to those found in Americans living in Jakarta. Preliminary results of host preference feeding experiments by anophelines at the study site did not suggest that this phenomenon would explain the observations. Humoral immune responsiveness to falciparum malaria appears to be age-dependent and chronic exposure-independent.

261 REACTIVITY OF SERA FROM A P. FALCIPARUM-P. VIVAX FOCUS IN INDONESIA WITH ERYTHROCYTE MEMBRANE ANTIGENS OF P. FALCIPARUM AND P. BRASILIANUM.

*A.J. Sulzer, R.A. Cantella, M.D. Clarke and W.P. Carney.
Malaria Branch, Centers for Disease Control, Atlanta, GA, USA; Departamento De Microbiologia, Universidad Peruana Cayetano Heredia, Lima, Peru; and Dept. of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA.

This study explored erythrocyte membrane antigens (EMA) of Plasmodium falciparum, P. malariae and of P. brasilianum, which is antigenically related to P. malariae. We used a battery of human serum samples from a focus in Indonesia of hyperendemic P. falciparum and P. vivax malaria, where P. malariae parasites were absent (as confirmed by blood-slide examination). In immunofluorescent antibody tests, 60 serum samples had titers of 1:256 to 1:16384 with P. falciparum and/or P. vivax and had minimal titers with P. malariae. Fifty (83%) samples reacted with ring-infected erythrocyte surface antigens (RESA) of P. falciparum, and 22 (44%) of those reacted with EMA of P. brasilianum. In earlier tests, we found that antibodies in serum specimens from people of a P. malariae and P. vivax focus, where P. falciparum was absent, reacted with RESA of P. falciparum. Of these specimens, 84% reacted with EMA of P. brasilianum, and 48% of those reacted with RESA of P. falciparum. When the eliciting species is P. falciparum about half of the specimens that react with RESA also react with EMA of P. brasilianum; when the eliciting species is P. malariae, the opposite occurs. With P. falciparum, EMA reacts with ring-stage parasites; membrane reactivity with P. brasilianum occurs with all intraerythrocytic stages. We conclude that P. falciparum and P. malariae infections elicit antibodies reactive with membrane antigens of erythrocytes infected with both P. falciparum and P. brasilianum.

ASTMH POSTER SESSION II

262 PRESENCE OF HISTIDINE RICH PROTEIN 2 (Pf HRP-2) IN THE SERA OF PEOPLE INFECTED WITH PLASMODIUM FALCIPARUM.

Marcela E. Parra and Diane W. Taylor, Department of Biology, Georgetown University, Washington, D.C. 20057.

Plasmodium falciparum synthesizes a group of proteins rich in histidine. One of these proteins, Histidine Rich Protein 2 (Pf HRP-2) has a molecular weight between 60-105 Kd depending on the parasite strain. By amino acid composition HRP-2 contains 34% histidine and 37% alanine. HRP-2 is synthesized throughout the erythrocytic cycle and is secreted into tissue culture supernatants. However, it has not been determined if PfHRP-2 circulates in the sera of people infected with P. falciparum and its significance in the host. In this paper we report the production of monoclonal antibody 1E-1 (mAb 1E-1) which binds to HRP-2. MAb 1E-1 was labeled with biotin and used in Western Blotting studies. Sera samples from Colombian adults with primary, secondary, and tertiary P. falciparum infections were evaluated. Each group consisted of sera from thirteen different individuals. As a negative control, sera from thirteen noninfected Colombians were used. Results showed that 38/39 samples from people infected with P. falciparum have circulating HRP-2. None of the sera from the control group evidenced circulating HRP-2. These results indicate that HRP-2 could be a candidate in diagnosing malaria in epidemiological studies.

263 IDENTIFICATION OF MALARIAL ANTIGENS IN THE URINE OF INDIVIDUALS WITH pLASMODIUM FALCIPARUM INFECTIONS.

Marina Rodriguez-del Valle and Diane W. Taylor, Department of Biology, Georgetown University, Washington, D.C., 20057.

Plasmodium falciparum antigens (Ags) can be found circulating in the sera or trapped in the form of immune complexes in different tissues of patients with malaria. We are interested in such Ags and in their potential for being excreted in the urine. Urine samples from malaria patients from Ghana and Thailand showed heavy proteinuria when studies by SDS-PAGE. BALB/c mice were immunized with a pool of urine from these infected patients, and anti-P. falciparum antibodies were detected following immunization. Spleen cells from immunized mice were used to produce monoclonal antibodies (MAbs). These MAbs detected proteins of molecular weights >200Kd and 35Kd by Western blot analysis in infected but not in uninfected people. MAb 1A10 was used to analyze urine and serum samples from the same individual. MAb 1A10 recognized proteins present both in urine and serum or in serum only. This study represents the first attempt to identify Ags in urine of malarial patients. Finding of Ags in the urine may serve as an alternative approach for the diagnosis of malaria and/or for a better understanding of renal pathology.

ASTMH POSTER SESSION II

264 NEOPTERIN AS A QUANTITATIVE MEASURE OF CELLULAR IMMUNE RESPONSE DURING ACUTE FALCIPARUM MALARIA

*A.E. Brown, P. Teja-Isavadharm, D. Keeratithakul, and H.K. Webster. U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Interferon-gamma (IFN) activated macrophages release a stable soluble metabolite, neopterin (Neop), which provides a quantifiable marker of T-lymphocyte activation. We have examined neopterin levels in Thai soldiers with acute malaria to better understand the kinetic characteristics of cellular immunity during malaria infection. Neopterin concentration was measured by HPLC of serial urine samples and expressed as $\mu\text{mol Neop/mol creatinine (Cr)}$. The study group consisted of 48 Thai soldiers with acute uncomplicated falciparum malaria. On the day of diagnosis, neopterin levels were elevated ($>300 \mu\text{mol Neop/mol Cr}$) in 44 (94%) of 47 subjects. The group mean ($\pm\text{SD}$) rose from 848 ± 425 on day 0 to 1206 ± 630 on day 3 and then fell to 543 ± 383 by day 7. Peak neopterin levels were higher in patients having 1° than 2° infections (1716 vs 1149 , $p=0.003$). Elevated serum IFN levels were associated with higher peak neopterin levels ($n=18$, $p=0.002$). In conclusion, neopterin measurements may prove useful in the study of malaria immunology by providing a quantitative marker of 1) an individual's level of acquired cellular immunity, and 2) the kinetics of T cell activation during infection and treatment.

265 PREVALENCE OF NATURALLY ACQUIRED ANTIBODIES TO A 43 AMINO ACID PEPTIDE DERIVED FROM PLASMODIUM FALCIPARUM ERYTHROCYTE BINDING ANTIGEN 175.

*B.K.L. Sim, H.C. Wynn, M. Carter, H. Marwoto++, and S.L. Hoffman+. WRAIR, Washington, DC; Johns Hopkins University, Baltimore, MD; +NMRI, Bethesda, MD, and USNAMRU-2 Detachment and ++National Institutes of Health Research and Development, Jakarta, Indonesia.

We have recently demonstrated that immunization of mice and rabbits with a 43 amino acid peptide (peptide 4) derived from the deduced amino acid sequence of a 175 KD P. falciparum erythrocyte binding antigen (EBA 175) induces antibodies that recognize merozoites by IFA and electron microscopy, and block parasite invasion of erythrocytes in vitro. To determine if natural exposure to malaria induced similar antibodies, we tested sera from residents of a village in Flores, Indonesia, where malaria is hyperendemic, for antibodies to peptide 4 by ELISA. The age specific prevalence of antibodies to peptide 4 was: 3-11 months (0/5), 1-4 years (0/8), 5-9 years (4/12), 10-14 years (8/11), 15-19 years (3/7), 20-29 years (7/13), 30 or greater years (4/16). Seven of the 10 highest levels of antibodies to peptide 4 were found in sera from individuals aged 7-13. These data clearly demonstrate that natural exposure to malaria induces antibodies to a defined epitope on EBA 175. The limited data acquired thus far suggest that, under these conditions of malaria transmission, the antibody response to this peptide peaks in early adolescence, several years after the prevalence of malaria in the population begins to fall, and then drops with malaria prevalence later in life.

ASTMH POSTER SESSION II

266 ANTIBODY TO THE RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN (RESA): PREDICTIVE VALUE OF PROTECTION IN A RURAL COMMUNITY IN MADAGASCAR.

*P. Astagneau, J.P. Lepers, C. Chougnet, C. Gaudebout, I. Hatin, E. Bailly, P. Coulanges, P. Deloron. INSERM Unité 13, 75019, Paris, France and Institut Pasteur de Madagascar, Antananarivo.

To investigate the role of antibodies to RESA under conditions of natural exposure to *P. falciparum*, we conducted a prospective study in a rural community near Antananarivo, an area where falciparum malaria was reintroduced recently, after having disappeared for some 30 years. 83 subjects, 10 to 62 years old, were enrolled in an out-patients clinic and were followed for 20 weeks. Serum samples were obtained at enrolment for antibody measurement using a FAST-ELISA technique with 3 synthetic peptides representing the repeat sequences of RESA: a) (EENV)₅, b) (EENVEHDA)₄, c) (DDEHVEEPTVA)₂. Clinical and thick blood smear examination were performed weekly. Patients with a malaria attack received prompt treatment. Malaria attacks were defined as presence of a malaria parasite density either above 5000 per µl of blood or less with clinical symptoms. For each individual, the number of malaria attacks during the follow up was used as an indicator of his protection against malaria. At enrolment, 49% of subjects were infected with *P. falciparum*, with a mean parasite density of 7.341 per µl of blood. 38%, 73%, and 75% of individuals had antibody to (EENV)₅, (EENVEHDA)₄, and (DDEHVEEPTVA)₂, respectively. During the follow up, each individual presented 0 to 6 falciparum malaria attacks (mean ± SEM: 2.1 ± 0.25). Although falciparum malaria has reappeared only few years ago, the number of malaria attacks decreased with age. After age and others antibody level adjustment, the number of malaria attacks increased with the level of antibody to (DDEHVEEPTVA)₂. Conversely, both the parasite density at enrolment and the number of malaria attacks were negatively correlated with the level of antibody to (EENV)₅ or to (EENVEHDA)₄. Thus, the humoral immune response to the 8-mer repeat sequence of RESA may appear to contribute to the immune protection in malaria. The response against the 11-mer repeat sequence might be rather considered as an indicator of infection. These results underline that the various regions of the RESA molecule may elicit the production of antibody which exhibit different activities against *P. falciparum*.

267 IDENTIFICATION OF A 50 KD TRYPSIN FRAGMENT FROM PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES THAT BINDS TO THE CD36 MALARIA SEQUESTRATION RECEPTOR.

A.I. Meierovics, C.C. Magowan, N.N. Tandon, C.F. Ockenhouse, G.A. Jamieson, J.D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Sequestration of *P. falciparum*-infected erythrocytes (IRBC) along venular endothelium is critical for parasite survival and contributes to host pathology. The leukocyte differentiation antigen CD36 has been purified from platelets and shown to be a sequestration receptor for IRBC (Ockenhouse et al., Science 1989;243:1469). Binding of IRBC to CD36 can be prevented by treatment of IRBC with trypsin. To identify a trypsin-sensitive ligand, IRBC from a cytoadherent clone of the ITG strain were purified on percoll-sorbitol gradients, radio-iodinated, and treated with trypsin. The soluble trypsin fragments were collected and affinity-purified using CD36 coupled to CNBr-activated Sepharose. An iodinated trypsin fragment of approximately 50 kd bound specifically to CD36-Sepharose. Specificity of binding was demonstrated by the addition of unlabeled soluble CD36 which inhibited the binding to CD36-Sepharose, and by the absence of this 50 kd fragment in trypsin fragments prepared from uninfected erythrocytes. The 50 kd trypsin fragment may be the functional portion of a trypsin-sensitive, radio-iodinatable protein involved in cytoadherence. Further characterization of this fragment should lead to identification of relevant epitopes on the cytoadherence ligand that bind to the CD36 receptor.

ASTMH POSTER SESSION II

- 268 OUTBREAK OF ACUTE SCHISTOSOMIASIS IN A GROUP OF AMERICANS RETURNING FROM COTE D'IVOIRE. R.T. Bryan*, M.K. Michelson, M. Wilson, S. Wahlquist. Parasitic Diseases Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

Increasing numbers of Americans are travelling to areas of the world that are endemic for schistosomiasis. In March/April, 1989, 25 persons participated in a religious pilgrimage to Cote d'Ivoire. During their 3-6 week stay, 8 persons (6 men; 2 women) left the main group and traveled to a remote, rural area of western Cote d' Ivoire, a region endemic for both S. haematobium and S. mansoni. Seven of these 8 persons either waded or bathed in a local river. Within 4 weeks of their return to the United States, 6 of the 7 exposed persons developed clinical syndromes consistent with Katayama fever (fever, chills, gastrointestinal discomfort, and eosinophilia). Six persons (86%) were serologically positive for antibodies to S. mansoni; 4 of these 6 persons were parasitologically positive for eggs of S. mansoni. No infections with S. haematobium were detected. Four persons required hospitalization, but no serious complications occurred. All persons with confirmed schistosomiasis were treated with praziquantel; no severe side effects were noted. This is the second outbreak of schistosomiasis in American travelers in one six month period and it emphasizes the ongoing need for pre-travel health advice and continued awareness by US physicians of imported tropical infections.

NEONATAL MORTALITY ASSOCIATED WITH LOW BIRTH WEIGHT IN MALAWI.

- 269 *L. Slutsker, J.J. Wirima, C.O. Khoromana, and R.W. Steketee. Malaria Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; Ministry of Health, Lilongwe, Malawi.

Infants born to women in a study of malaria chemoprophylaxis during pregnancy were followed to assess neonatal death and its relationship to birth weight, prematurity (gestational age <37 weeks), and intrauterine growth retardation (IUGR, gestational age >36 weeks and birth weight less than 2500 g). Birth weight, gestational age, and occurrence of placental malaria infection were determined for hospital-born infants. 1600 infants were seen at least once; 60% were hospital-born, 17% had low birthweight (<2500 g) and 19% were delivered to mothers with a placental malaria infection. 70% of 74 neonatal deaths occurred within 7 days of birth. The overall neonatal mortality rate (NNM) was 6 deaths per 1000 live births. Low birth weight was associated with a nearly seven-fold increased risk of neonatal death (150 vs. 23 deaths per 1000 live births). Among the 227 low birth weight infants, one-third were premature and two-thirds had IUGR. The NNM for premature infants (211/1000 live births) was nearly 4 times that for infants with IUGR (59/1000). Although IUGR was twice as common as prematurity, it was associated with only 37% of neonatal deaths associated with low birth weight. Half of the observed neonatal deaths were associated with low birth weight. In rural Africa, interventions aimed at reducing incidence of IUGR, such as malaria chemoprophylaxis or nutritional supplements for pregnant women, will be only partially successful in reducing low birth weight. Supported by USAID PASA BAF 0421 PHC 22333.

ASTMH POSTER SESSION II

- SEVERITY OF ANEMIA IN ZAMBIAN CHILDREN WITH PLASMODIUM FALCIPARUM
 270 MALARIA. *T.L. Fisk, J.A. Wadje, I. Mbuze, E.B. Mvula, and G. Kakompe.
 Emory University School of Medicine, Atlanta, GA, USA, and Mukinge
 Hospital, Kasempa, Zambia.

To assess the contribution of Plasmodium falciparum to pediatric morbidity and mortality in rural Zambia, an investigation was conducted at a 170-bed mission hospital in April-May 1989. During an 8-week period, 2733 children (age 6 mos-6 yrs) were seen as outpatients, the major diagnoses being presumptive malaria (35%), slide-confirmed malaria (15%), and upper respiratory infections (18%); 206 children were admitted to the hospital, the majority (66%) with malaria. P. falciparum-related anemia was assessed by comparing 3 groups of 25 age- and sex-matched children: A, admitted to the hospital with slide-confirmed P. falciparum malaria; B, admitted with diagnoses other than malaria and blood smear negative; and C, asymptomatic controls. On admission (day 0) children in A (mean parasite density: 13440 parasites/ul blood) had more severe anemia (hematocrit $21.3 \pm 7.3\%$) than those in B ($28.9 \pm 6.8\%$). By day 3, the anemia in children in A had partially resolved; their hematocrit ($28.3 \pm 7.2\%$) was not significantly different from that in B ($28.9 \pm 5.2\%$). However by day 7 or day of discharge, both A and B still had hematocrits lower than C ($28.3 \pm 12.1\%$, $29.2 \pm 6.8\%$, and $33.0 \pm 3.8\%$, respectively). The improvement was less marked for children with parasite densities greater than 12000/ul blood (day 3 hematocrit: $24.3 \pm 10.5\%$). A blood transfusion was required for 4/25 children in A and 1/25 children in B. Thus, P. falciparum malaria contributes substantially to the spectrum of pediatric morbidity, and P. falciparum related anemia is an important cause for blood transfusions in this African hospital.

- FETAL WASTAGE IN MALAWI, AN AREA OF HIGH MALARIA ENDEMICITY.
 271 *J.G. Breman, R.W. Steketee, J. Wirima, D.L. Heymann, C. Khoromana.
 Malaria Branch, Division of Parasitic Diseases, Center for Infectious
 Diseases and International Health Program Office, Centers for Disease Control,
 Atlanta, GA, USA; Ministry of Health, Lilongwe, Malawi.

Fetal wastage (FW), defined as fetal death at ≤ 6 months gestation (nonviable abortion) or ≥ 7 months gestation (stillbirth) was examined among a cohort of women who attended any one of four antenatal clinics in Malawi. Women were enrolled in a malaria chemoprophylaxis trial, placed on one of three chloroquine regimens or on mefloquine, and followed through delivery. When delivery occurred at a hospital, gestational age and malaria infection of the mother and fetus were evaluated. Among 3088 women who gave birth, the incidence of FW was 54.4/1000 pregnancies; incidence of nonviable abortion and of stillbirth were 15.5 and 38.9/1000 pregnancies, respectively. Incidence of FW was independent of parity but was high for young age groups. Incidence was similar for the chloroquine- and mefloquine-treated groups. FW was significantly more common in women < 150 cm tall (relative risk, $RR=1.9$), women of < 50 kg body weight at enrollment ($RR=1.8$), and women who gave birth at home ($RR=2.1$). Maternal malaria infection measured at enrollment or at delivery was not associated with FW. Among identifiable risk factors for FW, malaria infection does not appear to contribute significantly to this adverse outcome in Malawi. Supported by USAID PASA BAF Q421 PHC 22333.

ASTMH POSTER SESSION II

- 272 CONGENITAL MALARIA: SPONTANEOUS POST-PARTUM CLEARANCE, MALAWI. *J.J. Wirima, D.L. Heymann, R.W. Steketee. Ministry of Health, Malawi; International Health Program Office and Malaria Branch, Centers for Disease Control, Atlanta, GA.

Congenital malaria infection, defined as parasites in the newborn's blood at birth, is thought to be an uncommon event in malaria endemic areas. We evaluated the presence of falciparum malaria in maternal peripheral blood (n=3797), placental blood (n=3918) and umbilical cord blood (n=3918) in an endemic malarious area in Malawi; malaria infection rates were 18.4%, 19.5% and 4.9%, respectively. Presence of maternal peripheral and placental infections and placental and cord blood infections were highly correlated, however, only 24.6% of cord blood smears were positive when placental infection was documented; this increased to >45% when placental malaria densities were ≥ 5000 parasites/mm³ of blood. Newborns with Plasmodium falciparum infection in cord blood were followed for up to 5 days for clearance of parasitemia. Among 13 newborns with cord blood parasites $>500/\text{mm}^3$, all cleared their parasitemia within 48 hours without antimalarial treatment. This finding, coupled with our previous observation that mothers also demonstrate rapid postpartum clearance of falciparum malaria, suggests that both mother and fetus/newborn have comparable immune functions which are capable of clearing falciparum parasites once the placental source of parasites is delivered. Supported by USAID PASA BAF 0421 PHC 22333.

- 273 FEVER AND MALARIA IN HOSPITALIZED CHILDREN, KINSHASA, ZAIRE. *K. Hedberg, N. Shaffer, F. Davachi, L. Bongo, K.M. Paluku, A. Vernon, P. Nguyen-Dinh, A.W. Hightower, and J.G. Breman. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA, USA; Mama Yemo Hospital and PEV/CCCD, Kinshasa, Zaire.

Plasmodium falciparum is endemic in urban central Africa, and is a major cause of severe illness in children. Since antimalarial drugs are widely used, aparasitemic febrile children are often diagnosed as having inadequately treated malaria. To determine causes of fever in hospitalized children and to identify factors that might distinguish malaria from other febrile illnesses, we evaluated 161 children with a history of fever who were admitted to Mama Yemo Hospital during November 1988. 83% were ≤ 5 years of age, and 67% had a history of cough in addition to fever. On physical examination, 75% were febrile ($>37.5^\circ\text{C}$), 45% had splenomegaly, and 36% had an abnormal mental status. 60% were parasitemic (GMPD = $14,928/\text{mm}^3$), 35% were severely anemic (hematocrit $<20\%$), and 7.5% had antibody to HIV. 82% had chloroquine or quinine detected in the blood. 16 (16%) of 98 blood cultures from febrile children yielded bacterial pathogens. Parasitemic children were more likely than aparasitemic children to have had splenomegaly (52% versus 34%, $p = 0.03$) and anemia (42% versus 24%, $p = 0.001$) but were less likely to have had cough (57% versus 82%, $p = 0.002$) or bacteremia (9% versus 26%, $p = 0.03$). These associations persisted in a logistic regression model that controlled for prior antimalarial treatment. Most febrile illness was associated with parasitemia; however, fever in aparasitemic children was often associated with bacteremia or respiratory symptoms. Careful assessment of febrile children is needed to ensure that appropriate antimalarial or antibacterial therapy is prescribed. (Supported by USAID PASA BAF 0421 PHC 22333.)

ASTMH POSTER SESSION II

- 274 ORAL CIPROFLOXACIN VS. CEFTRIAXONE FOR THE TREATMENT OF PENICILLIN-RESISTANT GONOCOCCAL URETHRITIS. J. P. Bryan*, S. K. Hira, W. Brady, C. Mwale, G. Mpoko, R. Krieg, e. Siwiwaliondo, C. Reichart, and P.L. Perine. Uniformed Services University of the Health Sciences, Bethesda MD and University Teaching Hospital, Lusaka, Zambia

Neisseria gonorrhoeae (GC) urethritis resistant to treatment with penicillin, tetracycline and/or spectinomycin are increasing in prevalence throughout the world. Few oral regimens are effective for treatment of resistant GC. In Zambia, 52% of GC isolates produced beta-lactamase in 1986. We conducted a prospective, double-blind, randomized clinical trial of oral ciprofloxacin (CIP) 250 mg vs. intramuscular ceftriaxone (CEF) 250 mg for treatment of uncomplicated gonococcal urethritis in adult males. 200 men were enrolled and treated. Both groups were comparable in age (27.5 years), prevalence of latent syphilis (14% and 10%), and HIV infection (32 and 38%). Of 165 patients with cultures positive for GC, CIP cured 83/83 (100%), including 26 with PPNG, and CEF cured 81/82 (98.7%) including 30 with PPNG. Both treatment regimens were well tolerated. *C. trachomatis* was present by DFA or culture in 10 (5%) participants. The GC isolates were equally sensitive to CIP and CEF with an MIC₉₀ of 0.01 µg/ml. 72% of non-PPNG isolates were resistant to Pen at ≥1 µg/ml and 50% were resistant to Tet at ≥ 4 µg/ml. No resistance to spectinomycin was seen. CIP is safe and effective therapy for uncomplicated GC urethritis, including that caused by PPNG and CMRNG.

- 275 PRECOOKED RICE POWDER ORS COMPARED TO STANDARD CITRATE-GLUCOSE ORS IN MAINTENANCE THERAPY OF REHYDRATED HOSPITALIZED CHOLERA PATIENTS IN JAKARTA. *N.H. Punjabi¹, C. Rasidi², S. Sundah², S.P. Pulungsih², M.A. Mochtar², N. Sukri¹, D.H. Burr¹, N.D. Witham¹ and F.P. Paleologo¹.
¹U.S. Naval Medical Research Unit No 2 Detachment, Jakarta, Indonesia;
²Infectious Diseases Hospital, Jakarta, Indonesia

The effectiveness of a pre-cooked rice powder oral rehydration solution (PR-ORS) was compared to that of the standard citrate-glucose oral rehydration solution (S-ORS) for maintenance therapy in 166 severely dehydrated adult cholera patients hospitalized at the Infectious Disease Hospital of Jakarta from AUG to DEC 88. All patients received initial rehydration over 6 hours with intravenous Ringer's lactate (RL) solution. Subsequently, they were randomized to receive either ORS. Due to severe ongoing fluid losses, 23 (13.9%) patients required intravenous reinfusion and were excluded from the analysis. The 73 patients who received PR-ORS and 70 patients who received S-ORS were comparable in age, sex, body weight, and frequency or duration of diarrhea and vomiting prior to admission. The average amount of pre-treatment RL infused was also comparable in the PR-ORS and S-ORS groups (8411 ± 1843 ml vs. 8345 ± 1767 ml), as was the duration of the diarrhea (36±12 hrs vs. 37±12 hrs). Comparing those treated with PR-ORS to those treated with S-ORS, the PR-ORS group had less stool output/kg BW (92±53 ml/kg BW vs. 145±121 ml/kg BW p<0.008) and less total ORS intake (8855±4510 ml vs. 12591±7879 ml, p<0.0002). Emesis in PR-ORS patients lasted slightly longer (4±8 hrs vs. 2±5 hrs, p<0.03) but the difference was not clinically significant. Treatment with PR-ORS appears to be better than S-ORS in reducing stool output and ORS requirements in hospitalized cholera patients.

ASTMH POSTER SESSION II

276 DOUBLE BLIND CONTROLLED TRIAL TO DETERMINE SIDE EFFECTS AND IMMUNO-GENICITY OF PARENTERAL Vi CAPSULAR POLYSACCHARIDE (CPS) TYPHOID VACCINE IN INDONESIAN CHILDREN AND ADULTS

*F.P. Paleologol¹, C.H. Simanjuntak², N.H. Punjabil¹, Hardjining, S.2, T. Pudjarwoto², and N.D. Witham¹

¹U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta, Indonesia;

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From 12 SEP to 14 NOV 1988, 258 Indonesian children (age ≤ 10 years and 31 adults participated in a double blind controlled trial to test the side effects and immunogenicity of the Vi CPS vaccine. Pneumococcal vaccine, another parenteral CPS vaccine, was the control. All participants randomly received one of the vaccines then, one month later, received the other. Side effects were recorded by questionnaire and examination 24 and 48 hours after parenteral vaccine administration. 199 individuals received Vi CPS vaccine as their first injection, while 100 participants received the pneumococcal vaccine. Overall, Vi CPS vaccination in children had less systemic and local side effects compared to pneumococcal vaccine. Side effects observed were a feeling of feverishness (4.8% vs. 14.8%, $p < 0.001$), headache (0.4% vs. 7.7%, $p < 0.0001$), decreased activity (1% vs. 4.6%, $p < 0.03$), pain on palpation (13.8% vs. 26.7%, $p < 0.001$) and increased skin temperature at the vaccination site (1.4% vs. 7.4%, $p < 0.001$). In children, symptoms appeared less prominent with the second vaccination when only headache, soreness and pain on palpation were significantly more common in the pneumococcal vaccine group. Except for a feeling of feverishness, similar symptoms were observed in adults, however, since the total number of adult participants was small, differences were not statistically significant. Results of immunogenicity assays will be presented.

277 CHLOROQUINE DOES NOT AFFECT ANTIBODY RESPONSE TO YELLOW FEVER VACCINE. Barry M, Patterson J, Ratcliff B, Tirrell S, Shope R. Department of Medicine, Yale Arbovirus Research Unit, Yale University School of Medicine.

Chloroquine prophylaxis for malaria has been associated with hyporesponsiveness to rabies vaccine. Experimental data indicate that chloroquine inhibits replication of yellow fever (YF) *in vitro* yet there has been no clinical evidence to support that antibody response is adversely affected by chloroquine. Sixty-one volunteers took part in a prospective study of the effect of chloroquine on development of neutralizing antibody to YF 17D vaccine (Lot 8C91167). Six of these had pre-existing flavivirus antibody and are not included in the analysis. Each test subject received chloroquine phosphate 500 mg at the time of vaccination and at weekly intervals for 4 additional weeks; each control subject received vaccine alone. The vaccine titrated 5.1 log ID₅₀/ml in 3 day old mice. Serum plaque reduction neutralization antibodies were determined on days 0, 14, 35, and 210. There was no difference between the test and control groups in response rate as defined by peak antibody titer $\geq 1:10$ (test=64.3%, control=66.7%, $\chi^2=0.03$, $p>0.9$), peak geometric mean antibody titer (log geometric mean titer; test=1.73, control=1.80, $p>0.7$), day of initially detected antibody or time of peak antibody response. Sixteen subjects did not demonstrate antibody at the 1:10 dilution when the French neurotropic (FN) strain of YF virus was used in the test; however, when the 17D vaccine strain was used 10 of these had $\geq 1:10$ responses. Antibody testing with FN strain of YF may be a less sensitive method than testing with the 17D vaccine strain. There is no evidence that chloroquine adversely affects the antibody response to YF vaccine as measured by either test.

ASTMH POSTER SESSION II

- DETECTION OF MALARIA IN POPULATIONS WITH LOW-DENSITY
 278 PARASITEMIAS. *C. Wongsrichanalai, J. Pornsilapatip, V. Namsiripongpun,
 H. K. Webster, A. Lucchini, H. Wilde, M. Prasittisuk and S. Ketangsri.
 U.S. Army Medical Component, AFRIMS, Bangkok 10400, Thailand.

Detection of low parasitemia with Giemsa-thick smear (G-TS) requires experience and time and becomes impractical with high sample loads. Acridine orange fluorescent microscopy (AO/FM) of capillary centrifuged blood offers a technique with increased sensitivity that should permit efficient examination of large sample numbers. To evaluate the utility of AO/FM as an epidemiological tool we conducted a study in 293 asymptomatic villagers in endemic areas of southeastern Thailand. G-TS were prepared in triplicate and each set examined independently by an experienced microscopist (200 fields, x1000). Thus a G-TS reading of 600 fields was used as a standard for comparison with AO/FM. Capillary tubes coated with AO (QBCTM, Becton Dickinson) were prepared and examined for two minutes before declaring negative. Each AO/FM was done independently by two technicians unaware of the G-TS results. The number of G-TS positive was 27. Twenty-three (85%) of these were reported to be under 30 parasites/200 fields (or < 100 parasites/ul blood). Thirty-eight specimens were positive by AO/FM. Regardless of parasite species and stages, the AO/FM sensitivity was 63% (17/27) according to one technician and 70% (19/27) according to the other. Of 11 specimens positive by AO/FM but negative by G-TS, 2 were later confirmed by G-TS after examining > 200 additional fields. The test performed better for *P. falciparum* (PF) than for *P. vivax* (sensitivities: 81% [12/16] vs 45% [5/11]). Considering PF stages, AO/FM detected only one third of sexual PF but was 71% (10/14) sensitive for asexual PF. The overall test specificity was 96% (255/266). The levels of accuracy reported plus the advantages of examination time and ease of specimen preparation suggest that AO/FM, supplemented with G-TS to improve speciation and staging, is useful for screening malaria in populations with low parasite densities.

- CHLOROQUINE RESISTANT FALCIPARUM MALARIA ACQUIRED IN
 279 BURKINA FASO BY A BRITISH TRAVELER. M.S. Wolfe, A.M.J. Oduola, D.E.
 Kyle, L. Gerena, L.C. Patchen, and W.K. Milhous. Medical Service, Dept of
 of State and Experimental Therapeutics, WRAIR, Washington, DC & Malaria Branch,
 Centers for Disease Control, Atlanta, GA.

Nonimmune travelers frequently serve as sentinel cases in documenting the increasing prevalence and severity of chloroquine (CQ) resistance. A 50 y/o old British World Bank employee traveled to Burkina Faso July 6-18, 1988. He spent time in Ouagadougou where he recalled being bitten by mosquitoes at night and also traveled by car 10-12 July to/from Bobo Dioulasso. He returned by way of Abidjan, Ivory Coast, on 18-20 July but denied exposure to insect bites. Despite faithful compliance with weekly CQ prophylaxis, he developed chills, fever, headache, and sweats and sought medical attention in the United States on 27 July. Blood smears confirmed falciparum malaria and he was given a standard CQ treatment regimen. Fever and other symptoms persisted and he was subsequently admitted to George Washington University Hospital on August 2. Malaria smears were positive and CBC was normal except for a platelet count of 75,000. Acute hepatitis A infection was also diagnosed with elevated liver enzymes and positive anti-hepatitis A IGM. Treatment was with oral quinine sulfate was well tolerated and resulted in parasite clearance. He was discharged on August 6 and completed a 7 day course of tetracycline & 14 days of primaquine for radical cure of a possible mixed infection with *P. vivax* or *P. ovale*. Whole blood levels of CQ (548 ppb) and its metabolite, desethylchloroquine (DESCQ 193 ppb) confirmed therapeutic levels of drug. Malaria parasites were cultured *in vitro* and found to be resistant to CQ & DESCQ but susceptible to quinine, mefloquine, halofantrine, Paludrine® & Fansidar®. This case report and others currently under evaluation confirm the presence of drug resistant falciparum malaria in west Africa and serve to predict the clinical utility of other drugs in areas endemic for CQ resistant falciparum malaria.

ASTMH POSTER SESSION II

280 FECAL LEUKOCYTES: A SIMPLE MEANS FOR THE DIAGNOSIS AND MANAGEMENT OF DYSENTERY BY HEALTH CARE PROVIDERS IN EGYPT.

*N.A. El Masry, S. Bassily, and Z. Farid. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Oral rehydration therapy (ORT) has improved the clinical prognosis of patients experiencing severe episodes of acute diarrheal disease. Although ORT is accepted as appropriate therapy in most cases of watery diarrhea, patients with dysentery-type disease, particularly those with *Shigella* infection, may need supplemental antibiotics. In Egypt, where shigellosis remains a public health concern physicians are frequently faced with the challenge of diagnosis and treatment when laboratory support is unavailable. Thus, we evaluated the presence of fecal leukocytes (FL) in stools as a simple diagnostic aid for shigellosis. One hundred and nine (109) patients with acute diarrhea (>3BM/day conforming to the shape of the container) were studied. Forty-five (41%) of these patients presented with dysenteric symptoms (FL+) and 33 (29%) were culture confirmed shigella infections (primarily group B & D). The FL examination correctly identified 67% (22/33) of the shigella cases. In addition, this assay also ruled out shigellosis in 70% (53/76) of the culture-negative patients. The positive and negative predictive values of the FL test were 49% and 83% respectively. Based on these results, FL examination appears to have potential as a simple procedure to assist clinicians in making decisions concerning the use of supplemental antibiotics. Currently studies are underway to identify other clinical parameters that in combination with FL may result in a diagnostic algorithm with a better predictive value for shigellosis.

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TUBERCULOUS LYMPHADENITIS IN CAIRO, EGYPT.

281 * Z. Farid, M. Kamal, Y. Safwat, Z.A. Salama, and M. Kilpatrick. U.S. Naval Medical Research Unit No.3, Abbassia Fever Hospital, Ministry of Health, and Kasr El Aini Faculty of Medicine, Cairo University, Cairo, Egypt.

Of 80 patients with Fever of Unknown Origin (FUO) investigated in 1988, 41 had an infection, 23 a neoplasm and 16 a collagen-vascular disease. Of the 41 patients with an infection, 11 had tuberculous lymphadenitis. Nine were females and 2 were males and all except one female were young, aged 16 to 35 years. All were anemic and all had markedly elevated ESR and all except one patient had a strong tuberculin skin reaction. Sonographic and CT scan examination demonstrated widespread lymph node involvement in 6 of the 11 patients with extensive retroperitoneal lymph node enlargement in 4 and mediastinal adenopathy in 2 others. All 11 patients responded very rapidly to anti-tuberculous therapy and all became afebrile within 1 to 4 weeks of starting treatment. Six to 12 months later CT scan showed definite regression of retroperitoneal adenopathy in 2 patients. Sonographic and CT scan examination were of great value in improving diagnosis and in demonstrating the widespread lymph node involvement in these patients. Based on these observations, it is recommended that patients with suspect tuberculous lymphadenitis should be evaluated promptly by these techniques to aid in the early initiation of specific antibiotic therapy when indicated.

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ASTMH POSTER SESSION II

DEXAMETHASONE IN BACTERIAL MENINGITIS.

- 282 *N.I. Girgis, Z. Farid, I.A. Mikhail and R.L. Haberberger.
U.S. Naval Medical Research Unit No.3, Cairo, Egypt.

Four hundred and twenty nine patients with bacterial meningitis were assigned into one of two groups. Group I received Dexamethasone in addition to Ampicillin and Chloramphenicol, while those in group II received antibacterial chemotherapy alone. Ampicillin was administered in a dose of 160 mg/kg/day and Chloramphenicol in a dose of 100 mg/kg/day. Both drugs were given intramuscularly (IM) in 4 divided doses for a minimum of 8 days. Dexamethasone was given IM (8 mg to children below 12 years and 12 mg to adults) every 12 hours for 3 days. Both treatment groups were comparable with regards to age, sex, duration of symptoms and state of consciousness upon hospitalization. A significant reduction in mortality $P(<0.01)$ was observed in patients receiving Dexamethasone. The mortality rate was 9% with Dexamethasone and 20% in those patients who received only antibiotics. A reduction in neurological sequelae was also observed in patients receiving Dexamethasone, however, this reduction was only significant in patients with *S. pneumoniae* meningitis. Within this specific group none of the 36 patients who survived and received steroids had hearing loss while 3 of the 27 patients not receiving steroids became deaf $P(<0.001)$. No significant difference was observed between the two groups with regards to the time taken to become afebrile, regain consciousness, or in the mean admission and 24-36 hour CSF leukocyte count, glucose and protein content. It is apparent from this study that Dexamethasone is beneficial in reducing the morbidity and mortality in children and adults having bacterial meningitis.
(Supported by NMRDC, Bethesda, MD, Work Unit No. 3M464758.D849.BH.341).

EFFICACY OF MEFLOROQUINE AND CHLOROQUINE PROPHYLAXIS IN PREGNANCY.

- 283 *R.W. Steketee, J. Wirima, D.L. Heymann, C. Khoromana, J.G. Breman.
Malaria Branch, Division of Parasitic Diseases, Centers for Infectious Diseases, and International Health Program Office, Centers for Disease Control, Atlanta, GA, USA; Ministry of Health, Lilongwe, Malawi.

During pregnancy, antimalarial prophylaxis with chloroquine (CQ) (300 mg/week) in areas of CQ-resistant *Plasmodium falciparum* has been shown to have little effect on placental infection rates. In Malawi, from September 1987 through May 1989, we compared in pregnant women the efficacy of CQ and mefloquine (MQ) for the prevention of placental infection and low birth weight infants. For 1379 pregnant women, prophylaxis with one of the following drug regimens was supervised: 25 mg/kg CQ followed by 300 mg CQ weekly, 25 mg/kg CQ monthly, 300 mg CQ weekly, 750 mg MQ followed by 250 mg MQ weekly. The drug regimens were, respectively, 71%, 69%, 54%, and 96% effective in keeping women in their first or second pregnancy aparasitemic. Live, single infants, born to women who received CQ (n=1124) or MQ (n=255) or to 3023 women in a control group (who attended the antenatal clinic but did not receive supervised prophylaxis) were evaluated for placental infection and birth weight. Placental infection rates in the MQ group, the CQ group, and the control group were 6%, 29%, and 36%, respectively. The birth weights of babies born to women treated with MQ were significantly higher than those of babies born to women treated with CQ, whether firstborn or not. Effective prophylaxis should maintain the placenta free of parasites and be associated with normal birth weight; thus, MQ is more effective than CQ for pregnant women in Malawi. Supported by USAID PASA BAF 0421 PHC 22333.

ASTMH POSTER SESSION II

- 284 PRAZIQUANTEL TREATMENT FOR SCHISTOSOMIASIS CONTROL IN QALYUBIA GOVERNATE, EGYPT: 1976-1984. M. K. Michelson*, B. L. Cline, M. A. Habib, E. Ruiz-Tiben, F. M. Gamil, D. D. Juraneck, and H. C. Spencer. Parasitic Diseases Branch, CDC, Atlanta, GA; Dept. Trop. Med., Tulane University, New Orleans, LA; Center for Field and Applied Research, MOH, Cairo, EGYPT.

A 9-year longitudinal study (N=30,000) of praziquantel intervention for the control of S. mansoni (SM) and S. haematobium (SH) infections in Qalyubia Governate was completed in 1984. Prevalence and intensity of infection was assessed annually by Ritchie exams and urine sedimentation/filtration on the same 25% sample of all households. Treatment, single dose praziquantel (40 mg/kg), was given on the basis of Kato exams and urine sedimentation performed on all villagers every 2-3 years. Prevalence of SH declined from 18% to 0% in the study villages. However, a concurrent decline in prevalence from 42% to 7% was observed in the control village. The prevalence of SM declined from 53% to 38% after treatment compared to an increase in prevalence from 50% to 65% in the control village. High-intensity infections decreased from 2% to 0% for SH and from 9% to 5% for SM. These results confirm earlier reports that SH is spontaneously disappearing from this region. The finding that praziquantel decreases prevalence and intensity of SM infections, among the most heavily infected, supports the utility of this type of intervention. These findings are of relevance in formulating current schistosomiasis control strategies in Egypt.

- 285 BIONOMICS OF CANOPY MOSQUITOES IN A TROPICAL FOREST. John L. Petersen* Gorgas Memorial Laboratory, Panama.

Dry season survival of Flaviviruses in the tropics is poorly understood. A hypothesis to explain long-term maintenance of St. Louis Encephalitis virus (SLE) and yellow fever virus (YF) is the sequestering of these viruses in adult mosquitoes that remain active throughout the dry season. Since 1972, workers at the Gorgas Memorial Laboratory have monitored canopy mosquitoes of the genus Sabethes at the Maje Island Scientific Reserve, a lowland tropical forest enzootic for SLE and a corridor for YF activity. Techniques employed included human bait catch, sweep net, animal-baited traps and oviposition traps set at ground-level and canopy-level. Significant seasonal changes in vertical stratification have been detected. During the 8 month rainy season (May-December), Sabethes species are restricted to the forest canopy. During the four month dry season (January-April) when strong winds blow through the canopy, these mosquitoes have been collected at ground level by human bait collections. Cage studies under semi-natural conditions at Maje showed that Sabethes cyaneus are long-lived mosquitoes that have a mean life span that extends throughout the dry season. Cage oviposition studies showed that hollow lianas are an attractive oviposition site for Sabethes tarsopus, a species whose natural breeding site remains unknown. Demonstration of year-round activity of adult Sabethes suggests that these mosquitoes could play an important role in dry season survival of Flaviviruses.

ASTMH POSTER SESSION II

PERSISTENCE OF MOSQUITO-BORNE ARBOVIRUSES IN KERN COUNTY.
286 CALIFORNIA, 1983-1987. W.K. Reisen, J.L. Hardy, W.C. Reeves, S.B. Presser, M.M. Milby and R.P. Meyer. Arbovirus Research Program, School of Public Health, University of California, Berkeley, CA 94720.

The seasonal dynamics of mosquitoes and their associated viruses were monitored longitudinally in mixed agricultural, marsh, riparian and foothill habitats to investigate the hypotheses that these viruses persist vertically by transovarial transmission and/or horizontally in secondary mosquito-vertebrate cycles. Western equine encephalomyelitis virus (WEE) was isolated frequently from adult females of the primary *Cx. tarsalis* and secondary *Ae. melanimon* vectors during the wet year of 1983 when 51% of 103 sentinel chickens seroconverted, but was not recovered from males or larvae and did not reappear during dry years or the subsequent wet year of 1986. None of 556 cottontail and jack rabbits collected during 1985 and 1986 had antibodies to WEE and WEE was not isolated from *Cs. inornata*. St. Louis encephalitis virus (SLE) was isolated infrequently from *Cx. tarsalis* females during the summers of 1983 and 1986 and once from males. In contrast, Hart Park virus (HP) was isolated frequently during summer from all habitats from *Cx. tarsalis* females, and once from males. Turlock virus (TUR) was recovered from adult female *Cx. tarsalis* during summer. California encephalitis virus (CE) was recovered during summer from adult females and males and larvae of *Ae. melanimon*, and rabbit seropositivity at a marsh increased from 23% in Apr to 74% by Oct. However, CE virus was not isolated from females collected during spring. Thus, WEE virus did not persist either vertically or horizontally and became extinct during periods of unfavorable weather. In contrast, CE virus (and perhaps HP and SLE) persisted by transovarial transmission; however, horizontal amplification during favorable weather periods seemed critical.

MODELING THE EXTRINSIC INCUBATION OF ARBOVIRUSES IN
287 MOSQUITOES IN KERN COUNTY CALIFORNIA.
R.P. Meyer*, J.L. Hardy and W.K. Reisen. School of Public Health, University of California, Berkeley, CA 94720.

Temperatures of mosquito microhabitats were measured by digital recorder at 3 locations in Kern County, California, USA. Thermal regimens representing the diurnal resting shelter and nocturnal air space (2 m above ground level) of *Culex tarsalis* were combined to produce an extrinsic incubation temperature model for arboviruses for each month of the year. Times of thermal transitions between the shelter and air space microhabitats were set to occur at egress (sunset) and ingress (sunrise), respectively. Temperatures measured by digital recorders compared favorably with 2 m air and sod temperatures measured concurrently by nearby California Irrigation Management Systems (CIMIS) weather stations. Monthly model means ranged from a low of 7.4 degrees C in Dec to a high of 21.5 degrees C in Aug. Air space temperature at egress seldom exceeded 25.0 degrees C during the summer and was above 5.0 degrees C at ingress during the winter. The overall composite thermal regimen depicted by the model was cooler than both the shelter and air space and was devoid of the extremes of the latter microhabitat. Within these limits, the extrinsic incubation of either WEE or SLE viruses in susceptible *Cx. tarsalis* females would have occurred in a thermal regimen of least kinetic impetus for viral growth.

ASTMH POSTER SESSION II

- 288 MONITORING ENVIRONMENTAL PARAMETERS ASSOCIATED WITH THE FLOODING OF RIFT VALLEY FEVER VIRUS VECTOR MOSQUITO HABITATS IN KENYA WITH POLAR ORBITING METEOROLOGICAL SATELLITE DATA. K.J. Linthicum, C.L. Bailey, C.J. Tucker, K.D. Mitchell, T.M. Logan, F.G. Davies, D.J. Dohm,* C.W. Kamau, P.C. Thande, and J.N. Wagatoh. U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD; NASA, Goddard Space Flight Center, Greenbelt, MD; U.S. Army Medical Research Unit, Kenya; Veterinary Research Laboratory, Kenya.

Epizootics of Rift Valley fever (RVF) virus in Kenya are associated with rainfall patterns, produced by a strong intertropical convergence zone, that are both widespread and significantly above normal. This excessive rainfall is thought to precipitate RVF virus outbreaks and the subsequent transmission of the virus by flooding mosquito breeding habitats (dambos) and producing a hatch of primary (*Aedes* spp.) and secondary (*Culex* spp.) vectors. Ground moisture patterns, derived from measurements of green-leaf vegetation dynamics recorded by the advanced very high resolution radiometer instrument onboard National Oceanic and Atmospheric Administration satellites were used to observe rainfall patterns in Kenya and monitor resultant flooding of vector habitats. Satellite-derived data from mid-1981 to December 1988 have been analyzed with corresponding rainfall, flooding and vector population data as they relate to RVF virus ecology. Single (7 x 7 km) and multiple grid cell (200 x 300 km) analysis of vegetation data were used to quantify the potential for flooding of mosquito breeding habitats in small localized areas and large geographical regions, respectively. The ability to detect parameters accurately, such as rainfall and ground moisture, that determine flooding could provide local officials with sufficient warning to allow for implementation of specific control measures before a disease outbreak.

- 289 TRANSMISSION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY STRAINS OF AEDES ALBOPICTUS COLLECTED IN NORTH AND SOUTH AMERICA. J.R. Beaman* and M.J. Turell. Department of Arboviral Entomology, USAMRIID, Fort Detrick, Frederick, MD.

South American (Brazilian) strains of Aedes albopictus were significantly more competent vectors of Venezuelan equine encephalomyelitis (VEE) virus than were North American (United States) strains. Experimental studies were undertaken to ascertain the vector potential of North American (HOUSTON and ALSACE) and South American (SAO PAULO and SANTA TERESA) strains of Ae. albopictus for an epizootic (Trinidad donkey) strain of VEE virus. Infection rates were similar in all 4 strains of Ae. albopictus tested after ingestion of VEE virus from a viremic hamster. However, infection rates were directly related to the dose of virus ingested. Virus disseminated from the midgut to the hemocoel in about 80% of infected mosquitoes, regardless of the dose ingested or the time of extrinsic incubation. While all 4 strains of this mosquito transmitted VEE virus by bite to hamsters, transmission rates were significantly higher for the South American strains (31%, 54/163) than for the North American strains (7%, 17/250). Although VEE virus has never been isolated from Ae. albopictus, the introduction of this species into the Americas may allow it to play a role as an amplification vector in areas where epizootic strains of VEE are found or introduced.

ASTMH POSTER SESSION II

290 EFFECTS OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION ON THE BIOLOGY OF ITS ENZOOTIC MOSQUITO VECTOR, CULISETA MELANURA. *T.W.

Scott, S.C. Weaver, and L.H. Lorenz. Dept. of Entomology, University of Maryland, College Park, MD.

We recently reported that eastern equine encephalomyelitis virus (EEEV) causes cytopathology in the midgut of its North American enzootic mosquito vector, Culiseta melanura. This observation challenges previous beliefs regarding the benign nature of mosquito infection with arboviruses and led to studies reviewed below on the adverse effects of EEEV infection on Cs. melanura biology. Uninfected mosquitoes digested blood sooner than infected cohorts (mosquitoes containing blood by day of extrinsic incubation (EI) for uninfected vs infected: experiment 1, 0/6 vs 6/6 on day 4, 0/6 vs 2/6 on day 5; experiment 2, 3/20 vs 10/20 on day 3). Uninfected mosquitoes laid more egg rafts on the first day of oviposition (14 vs 6 and 23 vs 17). Later, days 8-10 of EI, infected mosquitoes laid more rafts (10 vs 3 and 16 vs 5). There was considerable variation and no difference in the number of 1st instar larvae that hatched from control vs infected mosquito egg rafts (mean progeny/raft: 65 vs 36 and 54 vs 44). EEEV infection reduced survival of mosquitoes maintained on a 5% sucrose diet. For example, there was 93% and 58% more mortality in the infected than control groups on day 28 of EI. Variation in temperature (15-20°C) did not eliminate this response. There were no differences in survival for mosquitoes maintained on 10% sucrose. These data suggest that EEEV-induced pathology affects Cs. melanura by protracting blood digestion, delaying oviposition, and increasing mortality. Results support the assertion that host-parasite evolution does not necessarily lead to benign, commensal relationships.

291 MODULATION OF ALPHAVIRAL REPLICATION BY AEDES ALBOPICTUS CELLS IN TISSUE CULTURE

E.J. Houk*, L.D. Kramer, J.L. Hardy and S.B. Presser. School of Public Health, University of California, Berkeley, CA.

Several colonized strains of the mosquito, Culex tarsalis, are able to modulate titers of western equine encephalomyelitis (WEE) virus and other alphaviruses after either peroral or parenteral infection. The present study examines whether modulation of alphaviruses can be demonstrated in vitro in mosquito cells.

Aedes albopictus (Singh) and Cx. tarsalis (Chao) cells were treated with transcription inhibitors, actinomycin D (actD) and α -amanitin (α A), and then, along with untreated cells, infected with WEE virus at an MOI of 0.1, 15 or 100. Titers of WEE virus measured at 24 and 48 hrs after infection of Cx. tarsalis cells were not significantly effected by inhibitor treatment. In contrast, viral titers in Ae. albopictus cells were increased as much as 1,000-fold by treatment with actD or α A. Similar experiments were done with 3 different lines of Ae. albopictus cells (i.e., U4.4, Singh and C6/36) and 3 alphaviruses [i.e., WEE, Sindbis (SIN) and Venezuelan equine encephalomyelitis (VEE)] and the rhabdovirus, vesicular stomatitis (VS). WEE, SIN and VEE, but not VS viral titers were increased by as much as 10,000-fold in U4.4 and Singh cells following treatment with α A. Modulation of WEE, SIN, VEE and VS viral titers was not observed in C6/36 cells.

These studies clearly indicate that the replication of alphaviruses can be modulated in vitro in mosquito cells and that modulation is controlled by a host cell protein(s). Attempts are being made currently to derive modulating and non-modulating clones of Singh cells for use in studies to determine the molecular basis of alphaviral modulation in mosquito cells.

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292 FURTHER CHARACTERIZATION OF THE *CULEX TARSALIS* MODEL FOR MODULATION OF WESTERN EQUINE ENCEPHALOMYELITIS (WEE) VIRUS

L.D. Kramer,* E.J. Houk, J.L. Hardy, and S.B. Presser, University of California, Berkeley, CA

Two genetic lines of *Cx. tarsalis*, high viral producers (HVP) and low viral producers (LVP), differ in the degree of replication of WEE virus by $10^{6.0}$ PFU following intrathoracic inoculation. These two lines have been further refined to produce an HVP line (L.H) congenic to the LVP line, differing only in the gene(s) controlling viral modulation. Using these lines of mosquitoes, we have been conducting experiments to characterize the mechanism for modulation of WEE virus.

The peroral ID_{50} of the HVP and L.H lines is approximately $10^{0.5}$, as compared to $>10^{4.3}$ for the LVP. Following peroral infection with $10^{5.0}$ PFU per mosquito, 40% of HVP and L.H females have mesenteron escape barriers; however, WEE virus grows to high titers in these same mosquitoes. This suggests that the mechanism for modulation of WEE virus is separate from that leading to virus entrapment in the mesenteron.

Inoculation of transcription inhibitors at the time of WEE infection results in viral titers in LVP equivalent to those in HVP mosquitoes. This suggests that LVP mosquitoes modulate by synthesizing a protein that interferes with the replication of WEE virus. Additional experiments are in progress.

293 SUSCEPTIBILITY OF *Aedes aegypti* TO DENGUE-1 IN PANAMA.

B.E. Dutary* and T. Solano. Gorgas Memorial Laboratory, Panama.

Panama City was reinfested with *Aedes aegypti* in July 1985 and by mid 1988 were found in every borough of the Metropolitan area. The origin of the mosquito has not been determined and therefore the vectorial capacity of this mosquito strain to dengue (DEN) virus is unknown. In October 1988, GML initiated susceptibility studies of Panama *Ae. aegypti* to DEN-1 which was selected because it is the virus present in the Caribbean and Central and South America. *Ae. aegypti* larvae were collected (1st-3rd instar) from four different city areas and maintained under standard laboratory conditions. Three days after emergence, females were fed on live mice, injected with three different high titered doses of DEN-1 (Jamaica strain-1977 passed 3 times in *Toxorhynchites*). The injected mice circulated 10, 100 and 1000 pfu/3µl during one hour. Each female engorged an average of 3µl of blood per meal, were divided in two groups and maintained at 37°C. The first group was harvested at day 7 post-feeding while group 2 was harvested on day 12 post-feeding. Head squash preparations were examined by direct fluorescent antibody test using monoclonal antibodies, while the thorax and abdomen were sonicated and inoculated into mosquito cell line (TRA-284) to observe cytopathic effects. The total number of females examined was 792; 7% (14/198) were positive after feeding on a mouse circulating 10 pfu/3µl of DEN-1, 7% (10/136) after feeding on 100 pfu/3µl infected blood and 46% (210/458) after feeding on 1000 pfu/3µl. The susceptibility of *Ae. aegypti* in Panama to DEN-1 virus will be compared to reports of the susceptibility of this mosquito in other countries.

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- 294 **ENDOPHILIC BEHAVIOR OF Aedes aegypti IN PUERTO RICO**
G.G. Clark,* H. Seda, and D.J. Gubler. Dengue Branch,
Division of Vector-Borne Viral Diseases, Center for
Infectious Diseases, Centers for Disease Control, GPO Box 4532,
San Juan, Puerto Rico 00936

A study of the resting behavior of Aedes aegypti in San Juan, Puerto Rico clearly demonstrated the endophilic nature of this species. It was undertaken after a series of ultralow volume (ULV) insecticide applications in urban areas of San Juan resulted in no impact on the wild mosquito population. An average of 17 to 33 females was collected with a backpack aspirator during weekly visits to houses in a lower middle class area of San Juan over a 52-week period. During 3 weeks in August and September, 1988, a period when increasing dengue transmission was detected in Puerto Rico, collections were made on 5 consecutive days and an average of 14 to 39 female Ae. aegypti was collected per house per day. Approximately 90 percent of these had recently taken a blood meal. If female bloodfeeding was evenly distributed between the 2 or 3 elderly occupants of these houses, from 7 to 13 bites per person per day would have occurred. Following the introduction of a dengue virus into an area with such high Ae. aegypti densities, rapid dissemination of the virus would likely ensue, unless a high level of immunity existed prior to its appearance. The endophilic behavior of this species clearly hampers the potential efficacy of ULV insecticide applications.

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LACROSSE VIRUS SMALL SEGMENT GENE EXPRESSION IN Aedes triseriatus MOSQUITO MIDGUTS.

*L.W. WASIELOSKI, B.J. Beaty, L.J. Chandler, M.J. Hewlett, Colorado State University, Fort Collins, CO, and University of Arizona, Tucson, AZ.

LaCrosse virus establishes a long term persistent infection in A. triseriatus mosquitos with no detectable CPE or untoward effect. We have previously reported reassortment of Bunyaviruses in dually infected mosquitos, but dual infection and subsequent segment reassortment was limited because of viral interference. The present study is an effort to better understand the phenomena of interference by elucidating the molecular events occurring from infection of the midgut to establishment of persistence. Mosquitos were infected per os with LAC bloodmeals. Midguts were dissected and pooled at various days post infection. The RNA was isolated using guanidinium thiocyanate extraction, and analyzed by northern and slot blot analysis with strand specific probes. The relative abundance of message, genome, and antigenome RNA's were quantitated. Persistent infection was correlated with a decrease in S mRNA levels.

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- 296 CALIFORNIA AND BUNYAMWERA SEROGROUP BUNYAVIRUSES FROM CALIFORNIA MOSQUITOES. G.L. Campbell*, B.F. Eldridge, J.L. Hardy, W.C. Reeves, and D.A. Dritz. University of California, Berkeley, CA, and University of California, Davis, CA.

As an initial study of the ecology of bunyaviruses in mountainous areas of California, more than 14,000 mosquitoes were collected as larvae and adults from the Sierra Nevada and Modoc Plateau regions during 1988, and assayed for viruses by plaque formation in Vero cells. Mosquitoes in the *Aedes communis* group of sibling species accounted for 90% of the total collection. Five virus strains were isolated, all from mosquitoes collected in Alpine County in the Sierra Nevada at approximately 2,300 meters in elevation. One strain was recovered from a pool of male *Aedes cataphylla* collected as larvae, which is evidence for vertical transmission. The remaining 4 virus strains were from pools of female *Aedes communis* (*sensu lato*) collected as adults. All 5 strains were shown to be California serogroup viruses closely related to Jamestown Canyon virus by cross-neutralization in Vero cells.

A recent serosurvey of deer in California revealed widespread evidence of the presence in both low and high elevation regions of California of a Bunyamwera serogroup virus closely related to Northway virus. The previously known distribution of Northway virus was limited to boreal habitats in Alaska and Canada, where it has been shown to infect large mammals, including humans. Five strains of unidentified bunyaviruses that had been isolated from mosquitoes collected in Butte County in the Central Valley of California during 1970-71 showed close antigenic relationships to Northway virus by cross-neutralization. Four of these strains were isolated from *Anopheles freeborni* and 1 was from *Aedes sierrensis*.

- 297 DETECTION OF RIFT VALLEY FEVER VIRUS RNA IN PARAFFIN SECTIONS OF MOSQUITOES BY IN SITU HYBRIDIZATION. L.A. Patrican* and W.S. Romoser. Department of Zoological and Biomedical Sciences, Ohio University, Athens, Ohio.

Rift Valley fever (RVF) virus was isolated from 9/563 pools of *Aedes mcintoshi* (n=59644) as *Aedes lineatopennis* [Ludlow] during an interepizootic period in Kenya (Linthicum, Davies, Kairo & Bailey, 1985, J. Hyg. Camb. 95: 197-209). Although, *Ae. mcintoshi* has been implicated in RVF vertical transmission, our inability to colonize this species precludes us from demonstrating transovarial infection in the laboratory. We are currently using an in situ hybridization technique to identify RVF virus RNA in experimentally infected *Ae. mcintoshi* and *Culex pipiens*, an epizootic vector, to study the relationship between RVF virus infection and the potential for vertical transmission at the genomic level. A RVF virus cDNA probe labelled with digoxigenin dUTP is hybridized to cellular RNA in serial paraffin sections of mosquitoes. RNA-DNA hybrids are detected by enzyme-linked immunoassay using an anti-digoxigenin alkaline phosphatase conjugate and enzyme catalyzed color producing substrate. This technique has great potential in determining whether or not gene expression occurs in reproductive tissues and cells of *Ae. mcintoshi* and will hopefully provide insight into the capacity of *Ae. mcintoshi* to vertically transmit RVF virus. Comparisons with the avidin-biotin complex procedure used to locate RVF virus antigen in infected *Ae. mcintoshi* and *Cx. pipiens* with regard to viral morphogenesis and dissemination will also be presented.

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- 298 TRANSMISSION OF RIFT VALLEY FEVER VIRUS BY ADULT MOSQUITOES AFTER INGESTION OF VIRUS AS LARVAE. M.J. Turell*, K.J. Linthicum, and J.R. Beaman. Department of Arboviral Entomology, USAMRIID, Fort Detrick, Frederick, MD.

Mosquitoes exposed as larvae to a 0.1% liver suspension from a Rift Valley fever (RVF) virus-infected hamster became infected and transmitted RVF virus by bite to hamsters. As animals infected with RVF virus abort or die in the vicinity of larval breeding habitats, and infected tissue from these animals may be introduced into the water where mosquito larvae are developing, we investigated the potential for mosquitoes (Culex pipiens and Aedes mcintoshi), exposed as larvae to tissue from a RVF virus-infected hamster, to become infected, transstadially transmit virus to the adult, and for the adults to transmit virus by bite to hamsters. After exposure as larvae, 9% (5/54) of adult Cx. pipiens and 13% (17/128) of adult Ae. mcintoshi became infected. All of the infected Cx. pipiens and about half of the infected Ae. mcintoshi transmitted RVF virus by bite to hamsters. Because of its potential to infect large numbers of mosquitoes, ingestion of infected animal tissue may contribute to the explosive nature of some of the RVF outbreaks.

- 299 NONVASCULAR DELIVERY OF RIFT VALLEY FEVER VIRUS BY INFECTED MOSQUITOES. M.J. Turell*, A. Spielman, and R.A. Tammariello. Department of Arboviral Entomology, USAMRIID, Fort Detrick, Frederick, MD and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The primary site of Rift Valley fever (RVF) virus inoculation in vertebrates by a transmitting mosquito appears to be extravascular, rather than directly into the vascular system. To determine the route (extra- or intra-vascular) by which mosquitoes introduce virus when feeding on a susceptible vertebrate host, female Culex pipiens that had been inoculated with RVF virus were allowed to feed on the distal portion of the tails of 3- to 4-day-old suckling mice. The tails were then amputated at the midpoint at selected times (1, 5, 10, 30, 60, 120, and 240 minutes) after the 20-second feeding. Tails of about 20% of the mice in each litter were left intact as a positive control. Significantly ($p < 0.001$) fewer mice whose tails had been amputated < 10 minutes after mosquito feeding died (27%, 15/55) than did mice with intact tails (92%, 44/48). Although survival rates were not significantly higher ($p = 0.06$) if the tails were amputated ≥ 10 minutes after mosquito feeding than in those with intact tails, the median time to death was longer in the group with the amputated tails (53.5 hours) than in those with intact tails (46.0 hours). These studies indicate that mosquitoes deliver virus extra- rather than intra-vascularly when feeding on a vertebrate host. This should be taken into account in studies on viral pathogenesis.

ASTMH POSTER SESSION II

- 300 THE USE OF MOLECULAR GENETIC MARKERS TO ANALYZE
POPULATION GENETIC STRUCTURE OF THE VECTOR OF BLUETONGUE
VIRUS, CULICOIDES VARIIPENNIS.

W.J. Tabachnick* and W.C. Wilson. U.S. Department of
Agriculture, Agricultural Research Service, Arthropod-borne
Animal Diseases Research Laboratory, Laramie, WY.

Isozyme electrophoretic markers and DNA RFLPs are being studied to understand the population genetic structure of Culicoides variipennis. Field populations from throughout the U.S. have been analyzed for variation at 21 isozyme loci. Populations of C. variipennis exhibit substantially higher genetic heterozygosity than other insect disease vectors ($H_e = 0.170-0.210$), show consistent seasonal gene frequency changes at 2 loci (MDH and PGD), and are genetically differentiated into at least 2 subspecies, C. v. variipennis and C. v. sonorensis. C. variipennis genomic DNA has been cloned into the plasmid vector Puc 8. Over 200 recombinant DNA clones are being screened to identify appropriate DNA RFLP markers in the species for use in population genetic analyses. Results from both types of genetic markers are being used to understand vector competence of C. variipennis for bluetongue virus.

- 301 ISOLATION OF VIRUSES FROM CULICOIDES MIDGES DURING AN EPIZOOTIC OF
VESICULAR STOMATITIS NEW JERSEY, 1982. W.L. Kramer, R.H. Jones,
F.R. Holbrook, T.E. Walton, and C.H. Calisher. Nebraska Department of
Health, Lincoln, Nebraska; Arthropod-borne Animal Diseases Research Laboratory
USDA/ARS, Laramie, WY; and Centers for Disease Control, Ft. Collins, CO.

An arbovirus survey was conducted in Colorado and Utah during an epizootic of vesicular stomatitis New Jersey (VSNJ) that occurred in the western United States in 1982-83. From 120,422 insect specimens assayed, 106 viruses were isolated. Four were rhabdoviruses (VSNJ), 3 were orbiviruses (bluetongue serotype-11), 92 were Bunyamwera group (65 Main Drain and 27 Lokern), and 7 were Simbu group (Buttonwillow) bunyaviruses. Culicoides spp. accounted for 105 viral isolates (C. [Selfia] spp., C. variipennis [Coquillett], C. stellifer [Coquillett], and C. cockerellii [Coquillett]), and 1 was from Simulium bivittatum Malloch. C. (Selfia) spp. accounted for 59% of all pooled specimens and were the source of 89.6% (95) of the isolates. Insects from 2 sites accounted for 44% (52,802) of all pooled specimens and 67% (71) of the viral isolates. The isolations of VSNJ, Main Drain, Lokern, and Buttonwillow viruses from C. (Selfia) spp. are the first arbovirus isolations reported for this subgenus, and this is the first time these viruses have been isolated from Culicoides species other than C. variipennis.

ASTMH POSTER SESSION II

302 PREVALENCE OF ANTIBODIES TO FLAVIVIRUSES, SANDFLY
VIRUSES AND LEPTOSPIROSIS IN FEVER PATIENTS AT THE MILITARY
HOSPITAL, RAWALPINDI, PAKISTAN.

R.E. Krieg*, J.F. Duncan, J.P. Bryan, T.G. Ksiazek, J.W. LeDuc, B. Awan, A. Ahmed, M. Riaz, S. Nabi, P.L. Perine, L.L. Legters, M. Iqbal, and I.A. Malik. USUHS, Bethesda, MD, PULSE, AMC, Rawalpindi, Pakistan, and USAMRIID, Frederick, MD.

Adult males admitted to the Military Hospital with acute febrile illness, negative chest x-ray, negative smear for malaria and no meningeal signs were studied to determine the etiology of the illness. Approximately 33% of the fever patients had typhoid. Sera from the first 254 of the 683 patients identified from October 1986 to November 1988 were analyzed by enzyme linked immunosorbent assay (ELISA) for the presence of IgG and IgM antibodies to sandfly Naples (SFN) and Sicilian (SFS) viruses and IgG antibody to the flaviviruses, Japanese B encephalitis (JE) and Western Nile (WN) viruses. The sera were also tested by ELISA for the presence of IgG and IgM antibodies to leptospirosis. There were 93 patients (37%) with titers $\geq 1:400$ for JE and/or WN. The relative SFN titers of IgG and IgM suggest acute infection in 1 patient, 4 with recent infections, and 4 with remote infections. The patient with the apparent acute SFN infection had a positive blood culture for *Salmonella typhi*. Serology did not suggest acute SFS infections; however, three patients were probably recently infected. The IgG antibody prevalence rates for SFN and SFS were 37% and 52% respectively. The IgG prevalence rate for leptospirosis was 4.8%. Only one patient had antibody titers indicative of an acute infection. Infections with flaviviruses, SFN and SFS appear to be common, but were responsible for < 1% of acute febrile illnesses in this series.

303 SEROLOGIC PREVALENCE OF ARBOVIRUSES IN WHITE-TAILED DEER IN SOUTH
FLORIDA, 1984-1988.

*R.G. McLean, S.D. Wright, S.R. Ubico and D.J. Forrester. Centers for Disease Control, Fort Collins, CO and University of Florida, Gainesville, FL.

White-tailed deer (*Odocoileus virginianus*) were collected at numerous sites throughout south Florida during 1984-1988 to evaluate the health status of the deer population. A total of 227 deer sera were obtained and tested by the plaque-reduction neutralization test against 11 arboviruses. Serologic positive sera were found for 9 of the arboviruses tested with the highest prevalence occurring with Tensaw followed by the California group, Everglades, Vesicular stomatitis-New Jersey and Vesicular stomatitis-Indiana, eastern encephalitis, St. Louis encephalitis and Highlands J viruses. The spatial and temporal patterns for the various arbovirus infections in the deer are discussed.

ASTMH POSTER SESSION II

- 304 CHIKUNGUNYA VIRUS INFECTIONS IN PATIENTS AT SUMBER WARAS HOSPITAL, JAKARTA, INDONESIA, 1987-1988
C. Bartz¹, R. Tan¹, C. Maroef¹, A. Siel¹, H. Wulur², T.K. Samsi²
¹U.S. NAMRU-2, Jakarta Detachment, Jakarta, Indonesia, and ²Sumber Waras Hospital, Jakarta, Indonesia

A severe epidemic of dengue virus infection occurred in Jakarta, Indonesia in 1987-1988, when 1,716 patients presented to the Sumber Waras Hospital with suspected dengue hemorrhagic fever. Dengue infection was confirmed in 1,460 patients and of the remaining 356, twelve had chikungunya virus infection. Diagnosis was made either by isolation of an alphavirus which was indistinguishable antigenically from chikungunya virus or by a four-fold rise in hemagglutination inhibition serologic titer. TRA-284 cells and *Toxorhynchites splendens* were used for isolations. Those patients which had four-fold rises in titer to both alpha and flaviviruses were not diagnosed as alphavirus infections although some of these may be so. Five alphaviruses were isolated. All patients presented with fever, while five had a cough but only one complained of throat pain. Seven of the patients had a positive tourniquet test and one had excessive bleeding at venipuncture sites. Only one of the 12 experienced severe joint pain, the classical diagnostic sign of chikungunya infection. Leukopenia was seen in three patients, however, none of them became thrombocytopenic during their hospital stay. None of the patients developed shock.

- 305 IDENTIFICATION OF EPIDEMIC CHIKUNGUNYA (CHIK) IN NORTHEASTERN THAILAND
A. Nisalak*, A. Srisajjakul, K. Nakdi, S. Rojanasuphot, C. Hemachudha, N. Nutkumhang, N. Sahasakmontri and B. Innis. Dept of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok and Div of Epidemiology and Dept of Medical Sciences, Ministry of Public Health, Thailand.

From May to Nov 1988, there was a large increase in cases of fever and rash in NE Thailand (Surin Province) reported to the Ministry of Public Health. An investigation conducted in July 1988, in a single community (village A) experiencing typical illness, established that there was an ongoing epidemic of fever, acute joint and bone pain and rash among children and adults. Indices of *Aedes* larvae prevalence in village A were very high (Breteau index=203, house index=70). 85/342 (25%) members of 61 randomly selected households (representing 30% of village households) admitted having recent fever and joint pain or rash. Anti-CHIK IgM was detected in sera from 30/38 patients with the most recent onset of symptoms. The anti-CHIK IgM ELISA we employed had been carefully standardized with large numbers of control sera, including serial sera from experimentally infected monkeys. Reports of disease transmission in the district adjacent to village A prompted efforts to recover the etiologic agent. Between Sept and Nov 1988, 3 visits to 2 additional villages identified 34 patients with fever and acute joint pain or rash. Anti-CHIK IgM was detected in 14/21 acute sera. CHIK virus, identified by cross neutralization, was recovered from 3/23 acute sera cultured. There was little evidence of concurrent dengue transmission in any of the 3 villages studied, therefore we presume that most reported cases were CHIK infection. This is the first documented instance of widespread CHIK virus transmission in Thailand outside of Bangkok. Densities of mosquito vectors associated with endemic dengue transmission have the potential to introduce other arboviruses into Thailand communities.

ASTMH POSTER SESSION II

306 DENGUE FEVER: A REPORT ON THREE LABORATORY ACQUIRED INFECTIONS AT DIFFERENT INSTITUTIONS DURING 1988. M.P. Kiley,¹ L.M. Alderman,¹

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Dengue Fever is a significant disease in many tropical and subtropical areas of the world. The disease can present in a variety of forms from a very mild illness to the severe form, Dengue hemorrhagic fever. Because of great interest in the diseases caused by the virus, it is investigated in many laboratories throughout the world. The CDC/NIH guidelines recommend that Biosafety Level 2 practices be used while studying the virus in the laboratory. While approximately a dozen laboratory-acquired Dengue virus infections had been reported through 1986, we now report on three confirmed cases which occurred in 1988 alone. The first infection probably occurred during dissection of virus-infected mosquitoes, presumably because the procedure was done without benefit of gloves or mask. The second case appears to have been the result of contact with an aerosol produced within a faulty biosafety cabinet during preparation of suckling mouse brain antigen. Aerosol contact with improperly protected broken skin or respiratory exposure were the most likely cause of exposure. The third infection occurred while a technician was fixing infected mosquitoes and cut her finger on a glass bottle during the procedure. She continued with the procedure without benefit of gloves. Other possible sources of infection in this case were a mosquito bite or an aerosol infection from the fixation procedure. In each case the patients presented with an uncomplicated case of Dengue fever with an incubation period of 3 to 7 days. We will present the details surrounding each infection and review the containment levels and work practices recommended when working with this virus.

307 RECENT ISOLATIONS OF PUNTA TORO VIRUS FROM FEBRILE HUMANS IN PANAMA.

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A viral isolate was made from the acute phase serum of two U.S. Army soldiers with a febrile illness while stationed at Fort Sherman in the Republic of Panama. Viral isolate 86MSP16 was made from the blood of patient #1, drawn in July 1985. The second viral isolate was made from the blood of patient #2, drawn in September 1985. Both soldiers were staff members of the Jungle Operations Training Center when the onset of illness occurred. Each viral isolate was made initially in VERO cells, and subsequently identified as Punta Toro virus by fluorescent antibody tests and Punta Toro virus-specific monoclonal antibody. Viral identity was verified by plaque reduction neutralization test. These recent isolations of Punta Toro virus from febrile humans indicate that Punta Toro virus is still active in Panama.

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PHLEBOVIRUS INFECTIONS AMONG HUMANS WITH UNDIFFERENTIATED ACUTE FEBRILE ILLNESS, EGYPT, 1988-1989. M. Darwish¹, A. Zaki¹, D. M. Watts², T. G. Ksiazek³, and C. J. Peters³. ¹ Ain Shams University, Cairo, Egypt; ² U. S. Naval Medical Research Unit No. 3, Cairo, Egypt; and ³ U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21701

In 1988-89 acute and convalescent sera were obtained from patients in the Central Nile Delta who reported to an outpatient clinic with fever, malaise, and myalgia. Enzyme immunoassays (EIAs) for IgG antibody to sandfly fever Sicilian (SFS), sandfly fever Naples (SFN), and Rift Valley fever (RVF) viruses were performed on these sera. Analysis of acute and convalescent sera obtained from 45 patients by EIA and complement fixation tests indicated that the febrile illnesses experienced by 7 were caused by SFS and/or SFN viruses. Virus, identified as SFS, was isolated from the acute serum of 1 patient. An additional 21 patients were positive for either or both SFS and SFN viruses, but with less than a four-fold increase in EIA IgG titer. RVF IgG antibody was demonstrated in 11 of the 45 paired sera and IgM antibody was demonstrated in 8 of the 11 sera. However, there was no evidence of RVF seroconversion based on IgM titers, and 2 of the 3 patients whose IgG antibody titers increased by 4-fold or greater were among those which seroconverted to SFS and/or SFN viruses. Although IgM and IgG antibodies to RVF virus were demonstrated, results suggest that these antibodies resulted from recent infection by the other co-existing phleboviruses, SFS and SFN. Further IgM EIAs and neutralization tests for antibody to these viruses are now in progress and should greatly facilitate interpretation of the serology of SFS, SFN, and RVF viruses in patient populations in which multiple independent phlebovirus infections have occurred.

VIROLOGICAL AND SEROLOGICAL RESULTS FROM NATURAL AND EXPERIMENTAL INFECTIONS BY CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN WEST AFRICA.

309 Jean-Paul Gonzalez*, Bernard LeGuanno, Marc Guillaud, Jean-Paul Cornet, Jean-Louis Camicas, Jean-Pierre Digoutte and Mark L. Wilson. Institut Pasteur, Dakar, Senegal; Institut d'Elevage et de Medecine Veterinaire des Pays Tropicaux; Laboratoire ORSTOM de Zoologie medicale, USAMRIID, Ft. Detrick, Frederick, Maryland 21701.

We studied a fatal human case of Crimean-Congo Hemorrhagic Fever (CCHF) from southern Mauritania in 1988. Two other mild infections were also followed. CCHF infection was confirmed by virus isolation in each case. Antibody titers (ELISA) appeared to correlate inversely with severity of disease. The early development of IgM and IgG antibodies was documented. A survey of 1,283 sheep in Mauritania following the human case demonstrated an overall IgM antibody prevalence of 4.1%, with 17.7% IgG positive.

Experimental infection of sheep, laboratory rabbits, and chickens was undertaken to study the duration of detectable antibodies, and of CCHF virus infectivity to ticks. All species seroconverted, and virus was reisolated. Other West African vertebrates that are suspected in the natural cycle of CCHF were tested for their susceptibility to infection. *Mastomys* and *Arvicanthis*, two peri-domestic rodents were inoculated, as were guinea fowl and hedgehogs. No clinical symptoms were detected in any of these species; serological responses differed among the hosts, and virus was not reisolated. Attempts to infect *Hyalomma* and *Amblyomma* ticks feeding on certain of these animals were variable. We are systematically experimenting with indigenous vertebrate and tick species in order to define the potential reservoirs and vectors of semi-arid West Africa.

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- 310 **PREVALENCE OF JUNIN VIRUS (JV) ANTIGEN AND ANTIBODY AMONG RODENTS IN ENDEMIC AND NON-ENDEMIC AREAS OF ARGENTINE HEMORRHAGIC FEVER (AHF).**
 J. Mills, B. Ellis, J. Maiztegui, T. Ksiazek, *K. McKee, and J. Childs. Johns Hopkins University, Baltimore, MD; INEVH, Pergamino, Argentina; and USAMRIID, Ft. Detrick, Frederick, MD.

Epidemiologic studies of AHF in central Argentina define a circumscribed but progressively advancing zone for human disease. In conjunction with a field trial assessing the efficacy of a vaccine against AHF, we are conducting an extensive program of rodent trapping to monitor JV activity in high-incidence (currently active for disease), low-incidence ("historic"), and as yet uninvolved (beyond the advancing disease front) areas. Specimens obtained from rodents trapped in mark-release and removal grids were tested for the presence of JV antigen (Ag) and IgG antibody (Ab) by enzyme immunoassay (ELISA). Evidence for JV infection was found in 3% (45/1669) of rodents captured in high-incidence areas, 1% (3/460) from historic areas, and 2% (3/135) from sites outside the endemic zone. Most (61%) Ab-positive animals were Bolomys obscurus. In contrast, most Ag-positives (67%) were Calomys musculinus, although 15% were B. obscurus. Coincidence of Ag and Ab in the same animal was unusual (5% of positives). Patterns of Ag and Ab among these species suggest that C. musculinus probably maintains active virus infection allowing for potential transmission to other rodents or humans, while B. obscurus responds to infection by clearing JV and developing antibody. In addition, Ag and Ab were detected in 2 rodent species previously not reported as carriers for JV in nature (B. obscurus and Oxymycterus rufus).

- 311 **LIMITED PROTECTION AGAINST RABIES VIRUS CONFERRED BY IMMUNIZING MICE WITH CERTAIN OTHER RHABDOVIRUSES.**

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During studies to determine antigenic relationships between previously and newly recognized rhabdoviruses, we found that many were directly or indirectly related to rabies (Pasteur strain) virus. These included not only viruses belonging to the rabies serogroup, but also some formerly ungrouped viruses, and members of the Tibrogargan, Malakal, and bovine ephemeral fever serogroups. We questioned whether some of these viruses would protect mice against rabies virus. Therefore we immunized mice with representative rhabdoviruses by giving them 4 weekly i.p. doses of live virus, beginning when they were 5 weeks old. Serum samples were obtained when the mice were 10 weeks old and tested for neutralizing antibody to the immunizing virus. Irrespective of the results, the mice were challenged (footpad inoculation) with 100 10-week old mouse i.p. LD₅₀ of rabies (CVS strain) virus and observed for 21 days for signs of illness.

All mice immunized with rabies virus had neutralizing antibody to that virus and withstood challenge; mice administered PBS four times all died after challenge. Most (75-83%) mice immunized with viruses known to be closely related to rabies (Mokola, Lagos bat) were protected; certain other viruses appeared to confer protection to a lesser extent. Preliminary immunoprecipitation and SDS-PAGE analyses of viral proteins provided some molecular corroboration but did not correlate precisely with antigenic relationships.

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- 312 DECLINING HEPATITIS A ANTIBODY PREVALENCE AMONG CHILDREN IN THAILAND
 B.L. Innis, R. Snitbhan*, T. Laorakpongse, W. Munindhorn, S.
 Sriprapandh, C.H. Hoke. Dept of Virology, Armed Forces Research
 Institute of Medical Sciences, Bangkok and Ministry of Public Health, Thailand.

Hepatitis A (HAV) antibody prevalence rates measured in similar populations of Thai schoolchildren in a large city (Bangkok) in 1977 and 1987-88 and in a rural area (Kampangphet province; northern Thailand) in 1985 and 1989 were compared. Contemporary antibody prevalence among lower to middle class urban children, age 6 to 15 years, attending two public schools, was 2 fold to 8 fold lower than that measured 11 years ago. 61% of the 1987-88 subjects were attending the same school that was the site of the 1977 survey. No anti-HAV seroconversions were seen in 202 urban children followed for 10 months (Nov 87-Sep 88). Anti-HAV antibody prevalence rates for a random sample (n=1862 in 28 villages) taken from approximately 10,000 rural children were decreased 0.7 to 2 fold for ages 6-11 and unchanged for ages 12-13 when compared to rates calculated four years earlier for a random sample (n=879 in 12 villages) taken from approximately 65,000 children. The incidence of hepatitis A measured by seroconversion was 4.8% to 1.2% per year over the period 1985-87. In recent years, hepatitis A transmission in Thailand appears to have decreased, especially in Bangkok where 11% of the population resides. If this trend continues, increasing numbers of susceptible adolescents and young adults will be at risk for epidemic HAV. These circumstances may provide a potent argument for hepatitis A immunization programs in the future, if a safe and inexpensive vaccine becomes available.

- A WATERBORNE OUTBREAK OF LEPTOSPIROSIS AMONG U.S. MILITARY PERSONNEL IN
 313 OKINAWA, JAPAN.

*A. Corwin, A. Ryan, W. Bloys, R. Thomas, B. Deniega and D. Watts.
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 of Research, Washington, D.C. and U.S. Naval Medical Research Unit No.3,
 Cairo, Egypt.

A waterborne outbreak of leptospirosis occurred among U.S. military personnel during September 1987, on the island of Okinawa, Japan. Between 3 and 21 September 1987, 9 males averaging 22.5 years of age, were admitted to a military hospital with symptoms suggestive of leptospirosis. Acute sera were obtained and tested for leptospirosis by the microscopic agglutination test. Epidemiological findings yielded 2 case clusters distinguished by time and place of exposure. The overall case attack rate (AR) among the cluster of recreational swimmers was 466.6/1000 (7/15) with incubation ranging from 10 to 25 days (with a mean incubation period of 13.6 days). Two of the 7 persons who exhibited antibody to Leptospira consistent with acute infection reported no associated illness. The second cluster of cases had participated in combat skills training involving water related activities. An AR of 48.7/1000 (4/82 screened) was calculated. The incubation period ranged from 5-11 to 10-16 days (repeated water immersion was over a 7 day period and precluded an exact estimate of time of exposure). The lack of rainfall (less was recorded than for any period over the past 40 years) may have contributed to the 'risk of exposure' associated with this outbreak. Additionally, water immersion alone did not appear to result in leptospiral infection. However, inadvertent swallowing of water differentiated cases from noncases. (Partly supported by NAVMEDRSCHDEVCOM, Bethesda, MD, Work Unit No. 3M162770A870.AR.322)

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- 314 TIBERIAN ENVIRONMENT AND INFECTIONS: ANTIBODIES AGAINST HANTAVIRUS, LEPTOSPIRES, AND HEPATITIS A VIRUS IN EXPOSED PERSONS ON THE RIVER BANKS IN ROME.

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In Rome, human cases of leptospirosis among riverside inhabitants of the Tiber river have been recorded since 1922 as "Malattia dei Fiumaroli" (Tiber Fever). The first data on the presence of Hantavirus infection were obtained in 1984, when antibody was found in 2.5% of Rome residents. A strain of hepatitis A virus has been recently isolated from the Tiber river water. A survey of persons residing or working on the river banks, such as rodent control personnel, river policemen, diving firemen, boatmen, and shipyard workers, was undertaken to define the epidemiology of these infections in the Tiberian environment. None of the subjects at risk showed detectable antibodies to Hantaan or Seoul viruses, despite the presence of high number of rats infected by Hantaviruses (more than 50% of *R. norvegicus* were seropositive). Antibodies to *L. icterohaemorrhagiae* were found at rates ranging from 10% (boatmen) to 21% (trappers). Concerning the presence of antibodies against hepatitis A virus, the prevalence (50-60%) did not differ significantly from those observed in Central and Southern Italy among healthy inhabitants. Our survey confirms the presence of leptospirosis and hepatitis A infections in people living and working near the Tiber river. Surprisingly, despite the high prevalence and the very high antibody titer (1:4096) previously observed in rats trapped on the Tiber banks, none of the riverside workers at risk of exposure to wild rodents were infected with Hantaviruses, but they appear to be at high risk for leptospirosis infection.

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EPIDEMIC KERATOCONJUNCTIVITIS AT A U.S. MILITARY BASE: REPUBLIC OF THE PHILIPPINES

*S.F. Paparello, L.S. Rickman, H.N. Mesbahi, J.B. Ward, C.G. Hayes. National Naval Medical Center, Bethesda, MD, 13th Air Force Medical Center, Philippines, and Naval Medical Research Institute Unit No. 2, Manila.

Between August 1988 and January 1989, 2603 cases of acute conjunctivitis occurred at Clark Air Base in the Philippines. Clinical features of the disease were consistent with epidemic keratoconjunctivitis (EKC). Adenovirus types 19 and 8 were isolated from conjunctival swabs. Approximately 18% of 9167 active duty personnel were affected. In an attempt to contain the outbreak, active cases were isolated from the workplace resulting in 9038 personnel-days lost.

To evaluate the outbreak, data was obtained retrospectively by questionnaire from 338 cases and 404 controls. Forty-five cases were evaluated prospectively. Illness was characterized by conjunctival injection (100%), watery or mucoid discharge (98%), conjunctival follicles (91%), preauricular adenopathy (64%), bilateral involvement (53%), and punctate keratitis (27%). The mean age of cases was 26.3 (\pm 10.6 yrs). Cases were more common among those reporting: use of community bathrooms ($p < 0.025$); locker rooms ($p < 0.022$), towels ($p < 0.04$); sharing soap ($p < 0.001$); and intimate contact with a case. Wearing glasses or contact lenses, sharing of bedrooms, sharing utensils, or use of community swimming pools were not risk factors. The extensive loss of personnel days due to isolation of cases impacted adversely on military readiness. The future development of practical preventive measures without compromising military preparedness needs further study.

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- 316 Desenclos JC, MD, DFS/EPO/CDC, Department of Health and Rehabilitative Services 1317
Winewood Blvd, Tallahassee, FL 32301 Florida, Karl A Klontz, MD, MPH, Disease Control,
Florida Department of Health and Rehabilitative Services.

The risk of vibrio infection among raw oyster consumers in Florida

To estimate the risk of vibrio infection (VI) in the raw oyster (RO) eating Florida population in general and with or without a chronic liver disease, prior gastric surgery and diabetes we used data on cases of VI reported to the Florida Health Department from 1981 to 1988 and data on the prevalence of RO consumption in Florida obtained through the 1988 Florida Behavior Risk Factor Survey (BRFS). Information on chronic liver disease, gastric surgery and diabetes were collected for each case report of VI and during the 1988 BRFS. From 1981 to 1988, 315 symptomatic and laboratory confirmed cases of VI, aged over 15, were reported; 184 (58.4%) were associated with RO consumption 1 to 5 days prior to onset. Of those RO, related 22 (12%) died (V. cholera non-01: 1; V. parahaemolyticus: 2; V. vulnificus: 19). The prevalence among the Florida population aged 15 years or over in 1988 was 32.1 % for RO consumption, 2.4 for chronic liver disease, 2.3 for past gastric surgery and 5.3% for diabetes. The incidence of RO related VI was 7.7 per million population year in Florida. As compared to the non RO eating population RO consumers in Florida were 3.1 times more likely to have had a VI (range from 0.4 for V. alginolyticus to 15.1 for V. mimicus). Consumers of RO with a chronic liver disease had an incidence of VI 10.6 times greater as compared to RO consumers without chronic liver disease (range from 2.3 for V. cholera Non-01 to 51 for V. vulnificus). Similarly among RO consumers, those with prior gastric surgery had an incidence rate of VI 5.1 greater as compared to those without prior gastric surgery (range from 0 for V. parahaemolyticus to 18.6 for V. cholera Non-01). Diabetics had an incidence rate of VI 1.9 time greater as compared with non diabetics (range from 0.8 for V. cholera Non-01 to 3.1 for V. vulnificus). This study quantified the risk of VI among RO consumers in Florida and supports the hypothesis that chronic liver disease is a major risk factor for the occurrence of VI among RO consumers.

- 317 BACTERIAL PATHOGENS ASSOCIATED WITH INFECTIOUS DIARRHEA IN DJIBOUTI.
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U.S. Naval Medical Research Unit No.3, Cairo, Egypt; International
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Public Health, Djibouti.

A study designed to identify the bacterial pathogens associated with acute diarrhea and determine their antibiotic susceptibilities was carried out during February 1989 in and around the east African City of Djibouti. A total of 309 individuals belonging to mixed age-groups were investigated, 217 patients with diarrhea and 92 age-matched asymptomatic controls. Most enteric pathogens were isolated at surprisingly similar frequencies from both diarrheal and control stools: Enterotoxigenic E. coli from 11% of diarrheal stools vs. 11% of controls; Enteropathogenic E. coli from 9% vs. 13%; Enteroadherent E. coli from 11% vs. 14%; Salmonella spp. from 3% vs. 3%; and Campylobacter jejuni/coli from 4% vs. 4%. No enteroinvasive E. coli, enterohemorrhagic E. coli or Yersinia enterocolitica were recovered from either diarrhea patients or asymptomatic individuals. Only two bacterial enteropathogens, Shigella spp. and Aeromonas hydrophila were isolated exclusively from the diarrhea cases. These were isolated from 7% and 3% of the diarrheal stools respectively. Among the Shigella isolates, S. flexneri was the most common species (12/16). Multiple resistance to ampicillin, tetracycline and trimethoprim/sulfamethoxazole were found in most of the enteric pathogens isolated in this area, while all isolates were susceptible to norfloxacin. We conclude that in Djibouti in 1989, Shigella and Aeromonas should be considered as potential pathogens when isolated from diarrheic stools, and that antimicrobial agents should be chosen with care, when treatment is warranted for Shigella disease. (Supported by NMRDC, Bethesda, MD, Work Unit No. 3M162770A870.AR.322)

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- 318 EPIDEMIOLOGY OF HYDATIDOSIS IN THE WEST BANK: A RETROSPECTIVE STUDY.
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Hydatidosis is an endemic disease in many parts of the world, especially the Middle East region. This study is intended to examine the epidemiology of hydatid disease in the West Bank area. The retrospective part of this study has been initiated to evaluate the distribution of the disease by reviewing the surgical charts of infected patients. From 1981-1988, 111 surgeries were performed in 7 area hospitals with > 70 cases in the last 3 years. The cases involved mainly the liver (74), lungs (28) and other sites (21). The cases were reported for different age groups. The cases identified and operated upon were as young as 4 years of age and as old as 75 years of age. With respect to gender; women constituted 72% and men 28% of the cases. The location of the cysts with respect to age groups showed that both the liver and the lungs were equally involved for individuals of less than 20 years of age and thereafter, more liver cysts were reported. Solitary cysts formed 59% of the cases while multiple cysts occurred in 41% of the cases. One district in the West Bank (Hebron district) has 40% of the cases. This district is known to be more of a rural and agricultural area. Herd raising of sheep and goats is a common means of livelihood. Recurrence has been reported in 13% of the cases reviewed. From these findings, it is shown that the West Bank area includes endemic foci of hydatidosis. However, the figures reported in this study are conservative ones due to several factors. A prospective study is needed to identify new cases by applying serological as well as radiological procedures from the endemic areas.

- 319 AN UNPRECEDENTED EPIDEMIC OF MALARIA IN THE REPUBLIC OF DJIBOUTI DURING THE WINTER SEASON 1988/89.
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Falciparum malaria has occurred only sporadically in the east African country of Djibouti and has never been considered to be a public health problem. The combination of extremely high outdoor temperatures with little yearly rainfall and few bodies of surface-water suitable as breeding sites was believed to be responsible for the lack of completion of the parasite's life cycle and the inadequate development of the mosquito's larval stage. Beginning in November 1988, epidemic numbers of malaria cases were reported from the District of Dikhil, near the Ethiopian border, and from villages south of Djibouti City, close to the country's border with Somalia. In a subsequent survey comprising 174 individuals from these two areas, we were able to confirm that 29 (39%) blood smears were positive for Plasmodium falciparum among 74 subjects with clinically suspected malaria. Eight blood smears were positive among 100 non-febrile controls ($P < 0.001$). Serum sporozoite antibodies were measured in all 174 study subjects in an effort to quantify the level of recent malaria exposure. Blood samples from febrile patients were inoculated into cell cultures in an attempt to rule out arboviral infections as the etiology of the fever. We conclude that in Djibouti in Winter 1988, an unprecedented epidemic of falciparum malaria occurred. This new malady for Djibouti may be related to the recent and successful agricultural projects expanding around the new water wells which are being drilled at selected dry river beds. (Supported by NAVMEDRSCHDEVCOM, Bethesda, MD, Work Unit No. 3M162770A870.AR.322)

ASTMH POSTER SESSION II

- 320 IMPORTED INTESTINAL PARASITE INFECTIONS: PREVALENCE IN CAMBODIAN REFUGEES SIX YEARS AFTER ARRIVAL IN CANADA.
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A follow-up study of 268 Cambodian refugees previously screened on arrival into Canada in 1982-83 was carried out between January and June 1989. In order to make direct comparisons between the previous and current studies, identical methods of stool collection and examination were followed. Two stool specimens were collected in SAF on two consecutive days and examined following ether concentration. The following prevalences were obtained: *Strongyloides* 13%, hookworm 17%, *Ascaris* 0%, *Opisthorchis* 5%, *Giardia* 2% and *E. histolytica* 2%. No statistical difference in *Strongyloides* prevalence was observed between 1982 and 1989 ($p > .85$). While the absence of *Ascaris* was expected, the continued presence of hookworm was not. Sub-group analyses examine issues of treatment and risk factors. Awareness of longterm infection with these pathogens in the Cambodian community has important implications in the clinical setting.

This research project was supported by the NHRDP of Health and Welfare Canada.

321 CELLULAR IMMUNE RESPONSES IN THE SMALL INTESTINE OF BALB/c MICE INFECTED WITH *CRYPTOSPORIDIUM PARVUM*.

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Cryptosporidium parvum, a coccidian protozoan, commonly causes a self-limiting diarrheal illness in humans and animals. Infection with this parasite in humans can lead to chronic diarrheal illness in the very young and immunocompromised. To date, little is known about the cellular immune response to *C. parvum* infections at the gut level. Immunohistochemical analysis of *Lyt-1*⁺ (T cell differentiation marker), *Lyt-2*⁺ (T cytotoxic/suppressor marker), and *Mac-2*⁺ (activated macrophage marker) cell populations were performed on excised ilea of naive (control) and infected BALB/c mice at varying time intervals following oral inoculation with 10⁵ *Cryptosporidium parvum* oocysts. Mice sacrificed at 8 days post-infection (PI) demonstrated a significant increase in *Lyt-1*⁺, *Lyt-2*⁺ and *Mac-2*⁺ cells (13 fold, 27 fold and 42 fold, respectively). At day 18 PI *Lyt-1*⁺, *Lyt-2*⁺ and *Mac-2*⁺ cell levels were still elevated (6 fold, 11 fold and 10 fold, respectively) in the infected mice compared to the control mice. Distribution of cells bearing the *Lyt-1*⁺ marker were evenly distributed throughout the lamina propria in the small intestine. Cells bearing the *Lyt-2*⁺ and *Mac-2*⁺ markers were concentrated in the apical portion of the lamina propria in close proximity to cryptosporidial development. The close proximity of expanded T-cell and macrophage populations to the villus surfaces where cryptosporidial development occurs may relate to immune events that have an influence on the outcome of this infection.

ASTMH POSTER SESSION II

322 CRYPTOSPORIDIUM PARVUM INFECTION IN CHILDREN OF MEXICO CITY.

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Cryptosporidiosis is now recognized as a leading cause of diarrheal illness among children of developing nations with prevalence varying between 1 and 30%. We performed fecal examinations from 350 children under 5 years of age presenting with diarrhea from June 1988 through May 1989 at 2 pediatric hospitals in Mexico City. Fecal samples were examined by using the Kinyoun-modified acid fast technique and by indirect immunofluorescence (IF) using anti-*Cryptosporidium* oocyst monoclonal antibodies (Merifluor *Cryptosporidium*TM). *C. parvum* was detected in 19/350 (5.43%) of the children by acid fast and in 25/350 (7.14%) of the children by IF. Of the 25 cryptosporidiosis cases, 17 were infants under 1 year of age and all had a mean of 8 stools per 24 hrs. All *Cryptosporidium*-positive samples also were examined to detect other enteropathogens. Enteropathogens were identified in 12/25 cases of cryptosporidiosis: 8 children had rotavirus, 4 had bacteria (*Salmonella* group B, *Escherichia coli*-012K71, *Campylobacter* spp., and *Pseudomonas aeruginosa*, respectively), and 2 of the 12 children also were infected with the helminth parasites *Ascaris lumbricoides* and *Taenia solium*. The prevalence of cryptosporidiosis in children with diarrhea in Mexico City as determined by IF, is comparable to other urban areas of developing countries. The prevalence was highest (11%) during the rainy summer months (June-September). The sensitivity and specificity of diagnosis using IF was higher than by using acid fast method.

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IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF CRYPTOSPORIDIUM ANTIGENIC SITES AND AN ASSESSMENT OF THE ROLE OF MONOCLONAL ANTIBODIES IN CONTROLLING CRYPTOSPORIDIOSIS.

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Relatively little is known about the antigenic relatedness of the different developmental stages of *Cryptosporidium* even though we possess a rather complete knowledge of the fine structural detail of all life cycle stages. Monoclonal antibodies (mAbs) were produced against *Cryptosporidium* by immunizing mice to be used in hybrid fusions with either purified sporozoites or unpurified merozoites. One mAb (Cmg-3), an IgG3, reacted with a 3.5 kd antigen of sporozoites in western blots. Another (C6B6), an IgG1, reacted with a 20 kd sporozoite antigen which was also strongly recognized by human, equine and bovine immune sera. Another monoclonal C4A1, an IgM, reacted with multiple sporozoite antigens above 25 kd. These mAbs showed similar (surface/cytoplasmic) immunoelectron microscopic colloidal gold labeling patterns with trophozoites, type I and type II meronts, microgametocytes, macrogametocytes, unsporulated and sporulated oocysts. Epitope sharing between *Cryptosporidium* life-cycle stages may have important implications in the biology and host-parasite relationship. These three mAbs were examined for potential modulation of cryptosporidial infections *in vivo* by daily oral mAb administration to oocyst-inoculated neonatal mice. Monoclonal-treated neonatal mice were sacrificed four and eight days post infection. Differences in infection rates were observed among the treatment groups. Suckling mice treated daily with orally administered mixtures of mAbs (ascitic fluid) showed significantly reduced parasite loads compared to control mice at four and eight days post infection while suckling mice receiving mAb Cmg-3 alone showed significant differences only at 4 days post infection.

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CRYPTOSPORIDIUM MURIS - LIKE OBJECTS FROM FECAL SAMPLES OF PERUVIANS.

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In 1985, Cryptosporidium muris-like objects were observed in the feces of a 65 year old woman suffering with severe chronic diarrhea. The oocyst-like objects were acid fast positive and measured 8µm in diameter. In 1987, an additional two patients presented with similar symptoms and similar acid fast objects. Endoscopy failed to demonstrate endogenously developing stages in these two patients. Microscopic examination of fecal specimens from infants enrolled in cohort studies involving Cryptosporidium parvum studies demonstrated 19 cases in which C. muris-like objects were identified in the fall of 1988 and an additional 31 cases in the fall of 1989. Diarrhea was associated with 23% of the individuals involved in these cases. The mean age to infection was 11 months and the mean duration of infection was 3 weeks. The oocyst-like objects weakly cross reacted with a commercially available Cryptosporidium specific monoclonal antibody (MAb) [Merifluor Cryptosporidium™]. They reacted more strongly with a Giardia specific MAb [Merifluor Giardia™]. Preliminary electron microscopy performed on samples concentrated using ethyl acetate suggests that the objects encountered may be cysts of an unidentified flagellate. To date, trophozoite forms have not been encountered.

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STATISTICAL ANALYSIS OF CLINICAL, IMMUNOLOGICAL, AND NUTRITIONAL FACTORS IN PEDIATRIC CRYPTOSPORIDIOSIS IN THE PHILIPPINES.

Daniilo M. Menorca, Marc A. Laxer, Alberto K. Alcantara, Marivyl Javato Laxer, Marcelino T. Fernando, and Virgilio Gonzales. U.S. NAMRU-2, Manila, Philippines, and San Lazaro Hospital, Manila Philippines.

The human immune response to Cryptosporidium is not well understood. Both humoral and cell mediated (CMI) mechanisms have been proposed, and there is evidence to support each. The purpose of this study was to conduct a statistical analysis of clinical and laboratory data collected during an investigation of pediatric cryptosporidiosis.

Serum antibody response to Cryptosporidium was determined by ELISA and the results reported as mean optical density of three trials per sample for IgA, IgG, and IgM. Total antibody levels were determined by radial immunodiffusion and reported as mg/dl. CMI status was evaluated by delayed-type hypersensitivity skin reaction. Serum iron and total iron binding capacity (TIBC) were measured by standard automated procedures. Nutritional status was determined by the method of Gomez.

A statistical analysis was performed by stepwise multiple regression, setting disease severity, expressed as days duration of diarrhea, as the dependent variable. The computer program (PRODAE) then selected significant independent variables by comparison to a set of pre-established criteria. On the first pass, TIBC was selected (P=0.0147) and on the second pass TIBC and age were co-selected (P=0.0005 and 0.0003 respectively). The significant role of iron and CMI in cryptosporidiosis are discussed.

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Swimming-Associated Cryptosporidiosis

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Between July 13 and August 12, 1988, 44 persons in 5 separate swimming groups developed a gastrointestinal illness following exposure to a Los Angeles swimming pool (pool A) in which a fecal accident had occurred during the first week of July. The overall attack rate was 72% (44/61) and 2 (4.6%) patients were hospitalized. Illness was characterized by watery diarrhea (88%), abdominal cramping (86%) and fever (60%) and was often protracted (median duration 5 days, range 1-30). The mean age of patients was 22 years (range 5-57); the male/female ratio was 1.2:1. Cryptosporidium was identified in stool specimens from 7 of 11 patients; no other enteric pathogens were identified. Among 32 elementary school students, illness was significantly more common among students using pool A than pool B ($P<.01$; $RR=8$, 95% CI 1.1, 56.8). Among the other 4 groups occurrence of diarrhea was significantly greater among individuals with exposure to pool A than among family members without such exposure. ($P<.001$; $RR=29$, 95% CI 7.4, 114.4). The attack rate was highest among those swimmers having heavy (≥ 3 hours per week) water exposure ($P=.001$; $RR 1.9$, 95% CI 1.3, 3.1). This outbreak demonstrates that Cryptosporidium may be acquired through recreational water contact. Resistance of Cryptosporidium to chlorination, a poorly maintained filtration system, continued pool use and possible ongoing contamination of the pool by infected individuals may have contributed to the size and extent of this outbreak.

- 327 A PROSPECTIVE STUDY OF THE TRANSMISSION OF TOXOPLASMA IN PANAMA J.K. Frenkel*, Rolando Saenz, Rosalia Quintero N., Lucia M. de Moreno, R. Centeno, Luis O. Perez, Ricardo J. Peart, Monica Sousa, Rebeca Galastica, Elizabeth Salas de Ortega, Suzanne Loo De Lao, Guillermo G. de Paredes. University of Kansas School of Medicine, Kansas City, Kansas, and Gorgas Memorial Laboratory, Panama City, Republic of Panama.

To determine the urban ecology of Toxoplasma and its transmission in Panama City, cats, soil, rodents, and birds are being studied, together with a cohort of approximately 500 one year old children. These will be followed for five years. The children are being tested serologically four times annually, using the direct agglutination test, the dye test, and where indicated the Immunosorbent agglutination assay (ISAGA) for IgM antibody. During an eighteen month period, nineteen seroconversions were observed in children who are being followed clinically and serologically. 60% of the mothers were seropositive (15-44, median 26 years). There were no difference in frequency of illnesses in the children of seropositive and seronegative mothers. Nevertheless, diarrheal diseases and asthma were twice as frequent in children of seropositive mothers (13 vs 7 and 18 vs 9) and bronchitis was twice as frequent in children of seronegative mothers (15 vs 6). 43% of 82 cats and 56% percent of 16 grackles (Cassidix mexicanus) were seropositive; toxoplasma was isolated from 2 of 50 rats and 2 of 179 house mice. No seropositivity or isolations have yet been found in 17 other ground feeding birds, 34 soil samples, or 25 fecal specimens of cats. Updated results will be presented in an attempt at explaining transmission. (Supported by NIH grant AI-23730)

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DIVERSITY OF rRNA SPECIES AND STRAINS OF *Pneumocystis carinii*

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18S ribosomal RNA genes of human and ferret *Pneumocystis carinii* have been cloned from infected tissue, lavage and sputum specimens and partially sequenced. Comparisons of the determined sequences reveal that all *P. carinii* 18S rRNAs are closely related to one another, and in turn, all related to the sequences of the fungi, *Schizosaccharomyces pombe* and *Schizophyllum commune*. *Pneumocystis carinii* rRNAs isolated from different host species exhibit distinctive patterns of sequence variation. Oligonucleotide probes specific for each type have been developed. Based on examination of several human lung biopsy or bronchial lavage samples, positive for *P. carinii*, presumed rat and ferret specific sequences have not been found. Too few host-specific isolates have been cloned and sequenced to assess whether particular biotypes may cross host boundaries.

From the lung tissue of one infected ferret, two different *P. carinii* 18S rRNA genes were isolated - one as the predominant species. *In situ* hybridization, using probes specific for each 18S type, clearly indicates a mixed infection in this animal.

329 PNEUMOCYSTIS AND TOXOPLASMA DIHYDROFOLATE REDUCTASES USED IN DRUG SCREENING: ACTIVITIES OF PYRIMETHAMINE ANALOGS

M. C. Broughton, M. S. Bartlett, and *S. F. Queener. Indiana
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The most successful drugs against both *Pneumocystis* (Pc) and *Toxoplasma* (Toxo) inhibit dihydrofolate reductase (DHFR): trimethoprim, pyrimethamine, trimetrexate. These drugs are limited either by low potency or by low selectivity, leading to toxicity. Therefore, analogs of these drugs are being screened against DHFR isolated from Pc harvested from rat lung or from Toxo harvested from mouse peritoneal cavities. Pc was prepared by homogenizing lung, filtering, and isolating intact organisms associated with lung cell plasma membranes. Toxo were harvested by repeated centrifugations and washings. Purified Pc or Toxo were disrupted by sonication; DHFR was assayed in the 100,000 X g supernate. IC₅₀ values are the micromolar concentrations of drug required for 50% inhibition in the assay, determined from inhibition curves using probit analysis. The ratio of IC₅₀ values for DHFR from rat liver (RL) and Pc or Toxo is >1 for agents selective toward Pc or Toxo.

Inhibitor	RL DHFR IC ₅₀	Pc DHFR IC ₅₀	Ratio	Toxo DHFR IC ₅₀	Ratio
Pyrimethamine	2.3	3.65 uM	0.6	1.17 uM	2.0
Metoprine	0.32	1.68	0.2	3.7	0.09
118203	42.5	85.1	0.5	2.4	17.7
125850	2.1	12.8	0.2	1.0	2.0
330465	18.9	2.8	6.8	0.35	54.0

The most promising compound in this series (330465) has a -N=N-N(Me)₂ group adjacent to the chlorine in pyrimethamine. These screens with specific drug targets are guiding selection of drugs for further testing in culture and animal models. (Supported by Contract N01-AI-87240, NIAID and NCI).

ASTMH POSTER SESSION II

330 FATTY ACID METABOLISM OF *PNEUMOCYSTIS CARINII* IN CULTURE

*J. R. Paulsrud, T. P. Glancy, M. M. Shaw, M. S. Bartlett,
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Total lipid fatty acid composition of *Pneumocystis carinii* trophozoites (PC) grown in culture (Bartlett, M.S. et al., Antimicrob. Ag. Chemother. 30:181, 1986) for up to 10 days was determined by gas-liquid chromatography (GLC) of the fatty acid methyl esters. Oleic acid percentages increased with time in the pellets from culture supernates and paralleled the increase in supernatant PC counts. Oleic acid did not increase in the presence of growth inhibitors such as trimethoprim/sulfamethoxazole. The percent oleic acid in growing cultures was 37.2% +/- 3.2% versus 18.3% +/- 2.1% in growth-inhibited cultures. A more sensitive and rapid analysis of PC metabolic activity was developed as a result of the GLC observations. C-14 labelled oleic acid was incubated with PC removed from wells or spinner flasks (Durkin, M.M. et al., J. Protozoa. 36:31, 1989) and incubated for 0 to 24 hours at 35C. Labelled PC were bound to Concanavalin-A linked to agarose beads, washed 3 times, and radioactivity determined by liquid scintillation spectrometry. The uptake for oleic acid was found to be linear to 24 hours of incubation. Incubation at 4C abolished uptake. This is a useful method to assess effect of antibiotics on PC metabolism. (Supported by NIH N01-AI-72647 and U01-AI-25859).

331 COMPARISON OF PARTICULATE 3,3',5,5'-TETRAMETHYLBENZIDINE AND 3,3'-DIAMINOBENZIDINE AS CHROMOGENIC SUBSTRATES FOR IMMUNOBLOT.

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In horseradish peroxidase (EC: 1.11.1.7) dependent immunoblot assays, particulate 3,3',5,5'-tetramethylbenzidine (TMB) is shown to be a more efficient immunoblot substrate than the standard substrate 3,3'-diaminobenzidine (DAB), because TMB is easily prepared, stable, and noncarcinogenic. Assays of antibody in a serially diluted human immunodeficiency virus (HIV) control serum (CDC reference CAT# VS2151) have the same sensitivity limit for both DAB and TMB (1:312,500). Complete, working substrate solutions of H₂O₂/TMB/enhancer and of H₂O₂/DAB were stored at room temperatures and at 48°C, respectively. Periodic tests showed the TMB substrate system to be functional after 4 weeks at 48°C and after 8 weeks at room temperature, while the DAB system was functional after 1 week at 48°C and after 4 weeks at room temperature. The stability, safety, and convenience of the commercially available TMB kits make this substrate ideal for immunoblot tests.

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332 RETROVIRAL SURVEILLANCE IN SOMALIA.

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Although bordered by countries with high prevalence of Human Immunodeficiency virus (HIV) infection, Somalia has been largely spared from the HIV pandemic. This survey was a continuation of an HIV surveillance program and was expanded to determine HTLV-1 prevalence.

During February-March 1989 samples were collected in the capital city of Mogadishu, Merka, Qoryoley and the coastal city of Kismayo near the Kenyan border. A total of 1200 sera were collected from the following groups: 68 prostitutes, 234 dermatology/STD patients, 122 TB patients, 52 patients from an infectious diseases ward and 809 subjects from the more general population. All sera were screened for HIV-1, HTLV-1 and syphilis. The prostitutes and any sera positive by HIV-1 ELISA were also screened for HIV-2.

Three sera were HIV-1 indeterminate, but none were confirmed positive. Eight sera were HTLV-1 positive (0.6%) with 20 giving indeterminate Western blots. HIV-2 assays yielded six indeterminates. Five of these were from prostitutes in Merka. In the entire study population 101/128 were FTA positive (7.9%). Infection among prostitutes was significantly more common with 29/68 FTA positive (42.6%, $p < 0.05$).

These data suggest that Somalia continues to have a low prevalence of HIV infection. HTLV-1 infection was found but, the prevalence is much lower than in endemic areas. The finding of five HIV-2 indeterminates in prostitutes in the isolated coastal town of Merka remains unexplained. Future studies will be required to monitor the HIV status of these individuals.

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333 HTLV-I INFECTION AMONG BLOOD DONORS, CHILDREN, AND HIGH RISK GROUPS IN EGYPT.

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Mohamed, N.M. Osman, D.M. Watts. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt, The Ministry of Health, Cairo, Egypt, and Al Azhar University, Cairo, Egypt.

Human T-cell lymphotropic virus type I (HTLV-I) has been associated with leukemia/lymphoma in many areas. Although the prevalence is high in some regions, the public health significance is not fully understood. Our objective was to assess the prevalence of HTLV-I infection in Egypt among normal adults and children, and young adults at risk for retroviral infections. A total of 1058 sera were screened for antibodies to HTLV-I using a commercial ELISA and confirmed by western blot (DuPont). Results indicated that none of the 411 normal children, 47 blood recipients, or 158 prostitutes were infected. In contrast, 3 of 133 (2.2%) blood donors, 2 of 279 (0.7%) drug addicts, and 1 of 30 (3.3%) patients with STD were confirmed positive. The prevalence of infection among all subjects was 0.5% (6/1058). There were 30 false positive reactors by ELISA, and 13 sera produced indeterminate results by western blot. There was no correlation between sex or geographic location of subjects and HTLV-I positivity. In comparison with HTLV-I seroprevalence in many parts of the World, the prevalence in Egypt is low, especially among high risk groups. (Supported by the NAVMEDRSCHDEVCOM, Bethesda, MD, Work Unit No. 3M463105H29.AA.335.)

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334 MECHANISMS RESPONSIBLE FOR THE DESTRUCTION OF *PLASMODIUM FALCIPARUM* BY ASCORBIC ACID AND COPPER

J. Golenser*, E. Marva*, A. Cohen*, M. Chevion**, Depts. *Parasitology and **Cellular Biochemistry, The Hebrew University, Jerusalem, Israel.

Ascorbic acid and copper caused a destructive effect on the in vitro development of *P. falciparum*. A synergistic effect of both reagents was demonstrated in particular when the parasites were grown in glucose-6-phosphate-dehydrogenase deficient erythrocytes. The present study evaluates mechanisms by which the parasite is affected.

The combination of ascorbate and copper exerted an oxidative stress and resulted in the release of oxygen reactive species (detected by electron spin resonance) which caused the oxidation of haemoglobin to methaemoglobin, the preoxidation of membrane lipids (as measured by the production of TBA-active substances) and could alter the profile of the proteins of erythrocytic membranes. Thus, the destruction of the parasite is probably the result of multiple modes of action.

"Free" plasmodia (released by saponin treatment from the host cell) were more vulnerable to the oxidative challenge than parasites within erythrocytes (viability was measured by hypoxanthine incorporation). This is in accord with the assumption that erythrocyte enzymes such as catalase, peroxidase and SOD provide some degree of protection to the intracellular parasite, from external oxidative challengers. The assumption that the damage was inflicted on the intraerythrocytic parasite by oxidative stress was further substantiated by the fact that the addition of extracellular catalase prevented the effects of ascorbate.

335 ANTIMALARIAL ACTIVITY OF PEPSTATIN A AND E 64 ON *P. FALCIPARUM* AND *P. YOELLI*. *E. Bailly, P. Deloron, I. Hatin, J. Savel and G. Jaureguiberry. INSERM U13, Hopital Claude Bernard, 75019 Paris, France.

Proteolysis is an essential mechanism of the intraerythrocytic development of malaria parasites. We previously characterized in *P. falciparum* two groups of proteases acting within an acidic pH range: one hemoglobinase with a cathepsin D-like activity and a cysteine protease. These proteases were strongly sensitive to Pepstatin A and E 64 (a cysteine protease inhibitor), respectively. The antimalarial activity of Pepstatin A was investigated in vitro on *P. falciparum*. 90% inhibition was obtained with 37 uM of Pepstatin A and the parasites were pyknotic form. Synchronized parasites were killed at the early trophozoite stage. E64 at 28 uM inhibited completely the in vitro growth of *P. falciparum*. Parasites exhibited morphological changes, schizonts maturation was stopped at the 2-8 nuclei stage. The difference observed in the inhibitory effects of Pepstatin A and E64 on *P. falciparum* might be related to a difference in function of cathepsin D-like protease and cysteine protease. We further investigated the effect of Pepstatin A in treating malaria in the white mouse experimentally infected with *P. yoelli*. A single injection (100mg/kg) failed to decrease parasitemia as compared to control. When the same dose was repeated 8 hourly for 24 hours, a 40% reduction of parasitemia was observed as compared to control, but did not last for a longer time than the treatment length. These preliminary experiments demonstrate that cathepsin D inhibitor (Pepstatin A) is active in vivo on *P. yoelli*.

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- 336 MEFLOQUINE CONCENTRATION IN WHOLE BLOOD AND URINE, AND ADVERSE DRUG REACTIONS IN HEALTHY VOLUNTEERS GIVEN A THERAPEUTIC DOSE OF MEFLOQUINE.
*L. Patchen, and S. Williams, Control Technology Branch, Centers for Disease Control, Atlanta, GA.

With the extension of chloroquine-resistant *Plasmodium falciparum* malaria, mefloquine (MQ) has become an alternate prophylactic and therapeutic drug. Studies of MQ treatment in Asia and Africa have reported cases of adverse drug reactions (ADR). With the recent licensure of MQ in the USA, a study was conducted to determine the concentration of MQ and its acid metabolite (MMQ) in whole blood (WB) and urine (UR), the applicability of UR field tests, and the association between MQ concentration in WB and severity of ADR. Healthy, non-parasitemic adult volunteers (3 male, 3 female) were given 15 mg/kg MQ orally. Samples were collected for WB (predose, +1,3,4,5,8 hr; +1,4,7,14,21,27,35,56,84 days) and UR (+1,7,14,21,35,56 days). Histories of ADR were recorded. MQ concentrations in WB and UR sampled at the same times were poorly correlated ($n=32$, $r^2=0.2998$), with UR and WB levels in the same range, suggesting that urine field tests for MQ will be of little use. Peak MQ-WB levels occurred at 8 hr and peak MMQ at 7-21 days, with large variations in metabolism among participants. Elimination $t_{1/2}$ ranged from 11-17 days. All participants had one or more ADR including light-headedness, vertigo, nausea, diarrhea, and vomiting; three had vertigo, nausea and/or diarrhea severe enough to be temporarily incapacitated. There were no significant differences in peak MQ-WB levels between those with incapacitating ADR ($X=1449$, $SD=298$) and mild ADR ($X=1730$, $SD=372$; $p=.383$). While this study does not support a correlation between high MQ-WB levels and severity of ADR, it does suggest this dosage is associated with substantial incidence of ADR, the severity of which is idiosyncratic. Controlled studies to clarify risk factors for ADR are indicated.

- 337 TREATMENT FOR AFRICAN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA IN AREAS OF CHLOROQUINE RESISTANCE: A COST-EFFECTIVENESS ANALYSIS.
*P. Sudre, J.G. Breman, D. McFarland. Malaria Branch, and International Health Program Office, Centers for Disease Control, Atlanta, GA,

In sub-Saharan Africa, *Plasmodium falciparum* infection is one of the leading causes of death in children less than 5 years of age. It is also the major cause of fever episodes in this age group. The current strategy to reduce malaria mortality is based on the prompt treatment of fever with chloroquine (CQ). Because *P.falciparum* resistance to CQ is spreading throughout Africa, comparisons of CQ, amodiaquine (AQ) and pyrimethamine/sulfadoxine (PS) as first-line treatment alternatives are needed. We analyzed a cost-effectiveness of these 3 drugs by using a model that considered compliance, drug efficacy, side effects, probability of infection, and case-fatality rate. Published data were used to determine the number of malaria-related fever episodes cured (once parasites cleared after treatment with a drug) and the number of malaria deaths prevented. We compared cost per death prevented by CQ, AQ, and PS at various levels of CQ resistance. PS and AQ are respectively 40% and 68% more expensive than CQ; they both cured more malaria episodes and prevented more malaria-related deaths than did CQ. CQ had the lowest cost per death prevented when the prevalence of RIII resistance was less than 20%. Potential lethal side effects of AQ and PS had a minor impact on comparative effectiveness. The model is useful to policymakers because it quantitates the variables essential to assessing a strategy to prevent malaria deaths by drug therapy. Supported by USAID PASA BAF 0421 PHC 22333.

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- 338 COMPARISON OF THE MULTIPLE DOSE KINETICS AND IN VITRO CHARACTERISTICS OF DAPSONE PLUS PROGUANIL VERSUS MALOPRIM.[®]
M.D. Edstein*, J.R. Veenendaal and K.H. Rieckmann. Army Malaria Research Unit, Milpo, Ingleburn, NSW, Australia.

Interest in the re-evaluation of antifolate combinations against chloroquine (CQ)-resistant falciparum malaria has prompted us to compare the multiple dose kinetics and in vitro characteristics of daily dapsone (DDS-10mg) plus proguanil (PROG-200mg) with weekly administration of Maloprim (DDS-100mg; pyrimethamine, PYR-12.5mg) in 6 healthy male volunteers. Steady-state drug concentrations were measured by HPLC. Kinetic parameters (mean values) were determined for DDS and PROG, respectively: maximum plasma concentration (C_{max})=285 and 151 ng/ml; elimination half-life (t_{1/2})=23 and 18 h, and clearance (Cl)=0.032 and 1.27 l/h/kg. Cycloguanil, the active metabolite of PROG, had a C_{max} of 56 ng/ml and a t_{1/2} of 15 h. After a washout period (>3 months) the same volunteers were administered Maloprim. Kinetic parameters were estimated for DDS and PYR, respectively: C_{max}=1,134 and 116 ng/ml; t_{1/2}=23 and 105 h, and Cl=0.038 and 0.016 l/h/kg. The drug combinations were assessed in vitro by measuring inhibition of re-invasion of 2 P. falciparum isolates (FCQ-27 and K1) grown in the presence of volunteer sera. Both FCQ-27 (CQ- and PYR-sensitive) and K1 (CQ- and PYR-resistant) isolates were completely inhibited by DDS plus PROG, whereas with DDS plus PYR only FCQ-27 was inhibited. The half-lives and in vitro findings suggest that DDS plus PROG may be a better matched and more effective combination than Maloprim against drug-resistant falciparum malaria.

- 339 PHARMACOKINETICS AND METABOLISM OF ARTEETHER IN THE ISOLATED PERFUSED RAT LIVER.
P.R. McNally, A.D. Theoharides, J.O. Peggins, B.G. Schuster, and T.G. Brewer*. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Disposition and metabolism of arteether, a new candidate antimalarial, was studied at two dose levels (5 and 10 mg/kg) in the isolated perfused rat liver using reverse phase high performance liquid chromatography with reductive electrochemical and radiochemical detection. The pharmacokinetic parameters of clearance, volume of distribution, mean residence time, and half-life were similar for the two groups. The hepatic clearance of arteether was rapid (18.0 ± 0.2 and 15.8 ± 0.01 ml/min) and the hepatic extraction coefficient was high, >0.90. Biliary elimination of [¹⁴C] labeled drug and metabolites was rapid (52 ± 12% of total dose at 90 min) and extensive (63 ± 4%, at 4 hr). The apparent volume of distribution of arteether (91 ± 0.02 and 86 ± 0.01 ml) was equal to the circulating volume (100 ml), less sampling volume (11 ml). Using bile hydrolyzed with aryl sulfatase and beta-glucuronidase, thermospray liquid chromatography-mass spectrometry confirmed dihydroqinghaosu as one of at least 13 putative biliary metabolites of arteether. In summary, the intrinsic hepatic clearance of arteether exceeds the contribution of hepatic blood flow, and arteether is rapidly metabolized and its metabolites are extensively cleared into the bile.

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- 340 IN VITRO INDUCTION OF RESISTANCE TO HALOFANTRINE IN CLONES OF *PLASMODIUM FALCIPARUM*. L. Gerena*, A.M.J. Oduola, B.G. Schuster, W.K. Milhous, and D.E. Kyle. Walter Reed Army Institute of Research, Washington, D.C.

The phenanthrene methanol halofantrine (HAL) is a new antimalarial drug developed at the Walter Reed Army Institute of Research which is now approved for use in France, Ivory Coast, Togo, and Congo. A primary concern with any new antimalarial drug is the possibility of clinically relevant cross-resistance to existing drugs such as mefloquine. In vitro induction of resistance to HAL has been accomplished in *P. falciparum* clones from Indochina (W2) and Brazil (311). Each clone was exposed to concentrations of HAL (2-12 ng/ml) increased stepwise over a period of seven months. In both clones increased resistance to HAL (3-10 fold), mefloquine (3-10 fold), and artemisinin (2.5-7 fold) was observed. In contrast, resistance to chloroquine decreased (up to 3 fold) as resistance to HAL increased. Resistance remained stable for up to 18 weeks after drug pressure was removed. Our results suggest that with the induction of resistance to HAL in vitro there may be a concomitant decrease in sensitivity to mefloquine and artemisinin and an increase in sensitivity to chloroquine. Epidemiological evidence suggests that HAL may be effective in some areas where multi-drug resistant *P. falciparum* occurs; however, the clinical efficacy of HAL against mefloquine treatment or prophylaxis failures remains to be evaluated.

- 341 IN VITRO EFFECTS OF PRIMAQUINE AND PRIMAQUINE METABOLITES ON EXOERYTHROCYTIC DEVELOPMENT OF *PLASMODIUM BERGHEI*. M.D. Bates, S.R. Meshnick, C.I. Sigler, P. Leland, D. Hayes, M. R. Hollingdale. Biomedical Research Institute, Rockville, MD; City University of New York Medical School, New York, NY.

The anti-malarial activities of primaquine and its metabolites against exoerythrocytic stages of *Plasmodium berghei* in vitro were compared with their abilities to spontaneously generate activated oxygen. A quantitative relationship between numbers of sporozoites added to cultures of hepatoma (HepG2-A16) cells and numbers of sporozoites produced was established, and the reduction in numbers of merozoites was used as an assay of drug effect. The ED₅₀ of primaquine was 3.7 to 3.9 X 10⁻⁶M. Several of the primaquine metabolites were much more potent than primaquine with ED₅₀'s as low as 2 X 10⁻⁷M. Metabolites containing the 4-amino-1-methylbutyl side chain were most effective in vitro. Superoxide generation was also measured for the various metabolites. Of the metabolites with 4-amino-1-methylbutyl sidechains, there was a correlation between superoxide generation and antimalarial activity. These results suggest that primaquine's antimalarial activity, like its hemolytic activity, may be mediated by activated oxygen.

ASTMH POSTER SESSION II

- 342 GENETIC LINKAGE OF CHLOROQUINE RESISTANCE, RAPID CHLOROQUINE EFFLUX, REDUCED CHLOROQUINE ACCUMULATION AND VERAPAMIL REVERSAL IN PLASMODIUM FALCIPARUM. I.Y. Gluzman*, D.J. Krogstad, L.J. Panton, and T.E. Welles. Washington University School of Medicine, St. Louis, MO and Laboratory of Parasitic Diseases, NIH, Bethesda, MD.

Chloroquine resistance in Plasmodium falciparum has been associated with decreased chloroquine accumulation; enhanced chloroquine efflux; with inhibition of chloroquine efflux by verapamil, diltiazem and vinblastine; with enhancement of chloroquine accumulation by these drugs; and with the reversal of chloroquine resistance by these drugs.

In these studies we examined the unique recombinant progeny of a cross between a chloroquine-susceptible (HB3) and a chloroquine-resistant (Dd2) clone of P. falciparum to determine whether these traits were linked to one another. The 7 chloroquine-susceptible progeny have initial efflux half-times of 50-70 minutes for chloroquine, the 8 chloroquine-resistant progeny have initial efflux half-times of 1-2 minutes. Verapamil actually reduced the chloroquine accumulation of the 7 susceptible progeny by 12%-27%, whereas verapamil enhanced the chloroquine accumulation of the 8 resistant progeny by 169%-403%. Verapamil had no effect on the chloroquine ED₅₀ with the 7 susceptible progeny, but reduced the chloroquine ED₅₀ 4- to 8- fold with the 8 resistant progeny. In contrast, hybridization with a probe for the P. falciparum P-glycoprotein gene correlated with neither susceptibility nor resistance to chloroquine.

These results suggest that chloroquine resistance, rapid chloroquine efflux, enhancement of chloroquine accumulation by verapamil, and the reversal of chloroquine resistance by verapamil are closely linked to one another and may be determined by a single gene.

- 343 ACCUMULATION OF CHLOROQUINE BY MEMBRANE PREPARATIONS FROM PLASMODIUM FALCIPARUM. *B.L. Herwaldt, P.H. Schlesinger and D.J. Krogstad, Washington University, St. Louis, MO.

In previous studies we have shown that chloroquine susceptibility and resistance are associated respectively with uptake and efflux of chloroquine by parasitized erythrocytes. We now have developed a membrane preparation from parasitized red cells and here present a characterization of chloroquine accumulation by that subcellular preparation. It displayed a time-dependent accumulation of ³H-chloroquine, which was inhibitable by excess unlabeled chloroquine. Accumulation was abolished by 0.05% Triton, but was enhanced by the use of a hypotonic medium, implying transport into vesicles, rather than simply drug binding to membranes. The accumulation required ATP, which could not be replaced with GTP, CTP, UTP or TTP. Accumulation was inhibited by N-ethylmaleimide and by reducing medium pH, whereas only high concentrations of vanadate inhibited accumulation. ATP-dependent acidification of this preparation was inferred from its NH₄Cl-reversible uptake of the fluorescent weak base acridine orange; but the weak bases NH₄Cl and propylamine only partially inhibited chloroquine accumulation. Verapamil and vinblastine, which inhibit efflux of chloroquine from resistant parasitized red cells, partially inhibited chloroquine accumulation by this subcellular preparation. We conclude that our membrane preparation contains vesicles that can be used as a model system to study the chloroquine uptake and efflux processes of the malaria parasite.

ASTMH POSTER SESSION II

- 344 SPERM COMPETITION IN THE DEER TICK IXODES DAMMINI: PROSPECTS FOR CONTROL BY STERILE MALE RELEASE.
B. Yuval and A. Spielman. Harvard School of Public Health, Boston, MA

We investigated the ecology and underlying physiology of the sexual behavior of the deer tick Ixodes dammini, vector of Lyme disease, in order to determine whether the abundance of this tick may effectively be reduced by releasing sterile males.

We determined when female deer ticks are inseminated in nature, how insemination affects feeding success, whether they can be inseminated repeatedly and whether particular sperm take precedence in fertilizing eggs.

Although spermatophores are present in about half of the questing female ticks, they are present in virtually all found on deer; the abundance of males on deer exceeds that of females. These ticks must be inseminated before commencing the rapid engorgement phase of feeding, but males need not be in attendance during feeding, provided that the female has been inseminated. Cobalt-irradiated males mate effectively. The fertility of eggs from females sequentially inseminated by irradiated and nonirradiated males, mainly reflected the last insemination.

We suggest that the abundance of these vector ticks may effectively be reduced by infesting deer with such irradiated male I. dammini.

- 345 EVALUATION OF BARRIER SPRAYING FOR THE CONTROL OF MALARIA IN THE DOMINICAN REPUBLIC.

*M.J. Perich, M.A. Tidwell, M.R. Sardelis, D.C. Williams, C.J. Pena, and L.R. Boobar. U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD; Vector Control Project Universidad Catolica Madre Maestra, Santiago, Dominican Republic; and International Center for Public Health Research, McClellanville, SC.

Efficacies of three classes of insecticides, organophosphate, carbamate and pyrethroid, as barrier sprays on various foliage types against malaria vectors were determined in the Laboratory. Results showed that both the organophosphate and carbamate insecticides provided significant control of the vectors for 3 weeks and the pyrethroid for a minimum of 8 weeks. As a follow up to laboratory testing, an aerial application of 1.2 g (AI)/ha deltamethrin around rural villages in southwestern Dominican Republic was evaluated as a barrier to malaria vectors using landing count and light trap data. The effect of this vector control methodology on the incidence of malaria was determined.

ASTMH POSTER SESSION II

346 TOXICITY EVALUATION OF METHOPRENE TO MOSQUITO LARVAE
AND NONTARGET ORGANISMS. Shobha Sriharan*, T.P.
Sriharan and Mary Thompson. Division of Natural
Sciences, Selma University, Selma, AL.

Methoprene, an insect growth regulator (IGR) is shown to be effective in controlling mosquito larvae and its persistence in water was reported for up to eight days. The evaluation of effective dose of this IGR on the larvae of mosquitoes, Aedes aegypti and A. albopictus and its toxicity to nontarget aquatic organisms has not been studied in detail. This paper describes: a) the effect of methoprene on the mosquito larvae; b) toxicity to nontarget aquatic organisms, Ceriodaphnia sp. (freshwater alga) and the fish, Fathead minnow; and c) persistence in treated water. To investigate the above, late instar (IV stage) of both the mosquito species, Ceriodaphnia sp. and Fathead minnow larvae were exposed to 0.33, 0.033, 0.0033, and 0.00033 ppm of methoprene. The residues in treated water were analyzed by chromatographic methods. The studies showed high mortality of the mosquito larvae at higher concentrations (0.33 and 0.033 ppm) and retardation in the larval development at lower concentrations (0.0033 and 0.00033 ppm) suggesting that this IGR blocks the metamorphosis. The Ceriodaphnia sp. was found to be sensitive even at the lowest concentration (0.00033 ppm). The toxicity to Fathead minnow larvae was observed at 0.0033 as well as 0.00033 ppm. Residues in water were present upto 7 days. Supported by NIH grant RR08169.

ASTMH POSTER SESSION II

348 VARIATION IN CUTICULAR HYDROCARBON PROFILES OF NORTH AMERICAN Aedes albopictus POPULATIONS.

*E.L. Kruger and C.D. Pappas. Science and Technology, Peru State College, Peru, NE.

Cuticular hydrocarbons were used as a means of determining geographic relatedness of several Aedes albopictus populations from North America. The geographic populations examined included those from Houston, Louisville, Chicago, Milford, Jacksonville, San Antonio, New Orleans, St. Louis, and Indianapolis. Cuticular hydrocarbons were extracted from ♂ and ♀ (in separate tests) specimens with hexane for analysis with gas chromatography-electron impact mass spectroscopy. Seventy-two cuticular hydrocarbons of Aedes albopictus were chemically characterized. Geographic populations of Aedes albopictus could be distinguished on the basis of 8 unique dimethyl compounds and 15 unique monomethyl compounds. Additionally, five n-alkanes will separate populations on the basis of relative abundance.

349 SPECIES DIVERSITY, BITING ACTIVITY AND FLAGELLATE INFECTION RATES OF PHLEBOTOMINE SAND FLIES COLLECTED AT TEKAL, GUATEMALA. ED Rowton*, CH Porter, RG Andre, TR Navin & JL Pozuelos.

Walter Reed Army Institute of Research, Washington, D. C., Centers For Disease Control, Atlanta, GA, Uniform Services University of Health Sciences, Bethesda, MD, Medical Entomology Research and Training Unit, Guatemala City, Guatemala.

Anthropophilic phlebotomine sand flies were surveyed at Tekal National Park in Guatemala during an outbreak of cutaneous leishmaniasis. Approximately half of 180 military troops stationed in the park acquired this disease during a one-year period. These soldiers lived in tents located on a cleared hilltop under tall trees, and the perimeter of their camp was cleared of underbrush for up to 100 M before dense jungle was encountered. Guard stations were located along this perimeter at ground level with the exception of one which was on a 3 M high platform close to the dense jungle canopy. All night sand fly collections were conducted over a two week period to identify those attracted to humans and to determine the flagellate infection rate. Landing captures of sand flies were made both at ground and canopy level. Lutzomyia panamensis and L. ovallesi each comprised about 35% of the collections. Other species collected were Lutzomyia ylephiletor, L. olmeca olmeca, L. cruciata cruciata and L. shannoni. More sand flies were collected in the jungle and on the platform station than at the cleared ground-level stations. High numbers (100 to >200 sand flies/hour) were collected from 2200 to 0300 hours. Lutzomyia ylephiletor was the only species found infected with flagellates. Its infection rate was 2% versus an overall rate of 0.18%. This is the first time infected sand flies have been collected off of soldiers serving on guard duty.

ASTMH POSTER SESSION II

350 FEMALE-SPECIFIC GENE EXPRESSION IN THE SALIVARY GLANDS OF MOSQUITOES.

*A.A. James, K. Blackmer, O. Marinotti, and G. Grossman. Harvard School of Public Health, Boston, MA.

We are developing methods for expressing genes in mosquitoes using endogenous control DNA sequences. Our approach includes the isolation and sequencing of genomic and cDNA clones, determination of their expression patterns through development and in tissues, and identifying the sequences that control the tissue-specific expression. Initially, the control sequences are linked to a marker gene to determine the specificity of expression of the hybrid gene. The marker gene is then replaced with a specific DNA fragment chosen to interfere with a specific pathogen. Two genes, D7 and G34, expressed specifically in the female salivary glands of adult *Aedes aegypti*, have been examined in detail. The putative promoter regions of these genes have been identified and are now the subject of a functional analysis using a somatic transformation assay.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN MIDDLE REPETITIVE DNA OF *AEDES ALBOPICTUS* DETECTED BY SOUTHERN BLOT HYBRIDIZATION.

*T.J. Monroe, J.O. Carlson, B.J. Beaty. Department of Microbiology. Colorado State University, Fort Collins, CO.

During the development of gene transfer systems in the mosquito, a moderately repeated DNA sequence was isolated and cloned from *Aedes albopictus* cells (clone C6/36). This 2kb fragment was used as a probe in southern blot hybridizations of genomic DNA from several geographically isolated populations of *Aedes albopictus* mosquitoes. This DNA element was found to be highly polymorphic not only between populations but also between individuals of the same population. The element has been further characterized by restriction mapping, northern blot, and sequence analysis.

An element such as this has potential use in determining geographical origins of disease carrying insects as well as a genetic marker for linkage studies. Sequence analysis is in progress and may elucidate the role of this repetitive element.

M: LATE BREAKERS IN MOLECULAR BIOLOGY WORKSHOP

352-359: The list of abstracts selected for the session will be available at the Registration Desk on Tuesday morning.

N: MOLECULAR VIROLOGY

360

GENOMIC AND ANTIGENIC COMPARISONS OF EASTERN EQUINE ENCEPHALITIS VIRUSES SUGGEST DIFFERENTIAL EVOLUTION OF NORTH AND SOUTH AMERICAN STRAINS. *P.M. Repik and J.M. Strizki. The Medical College of Pennsylvania, Philadelphia, PA.

As part of our effort to delineate critical elements involved in the ecology of eastern equine encephalitis (EEE) virus, we have investigated the genetic and antigenic relationships that exist between and among North and South American EEE virus isolates. More than 20 virus isolates from different geographic foci, collected over a span of 50 years and from various host species, were analysed. Oligonucleotide fingerprinting of genomic RNA revealed that all N. Am. isolates displayed a strikingly similar fingerprint pattern, with 72-98% oligonucleotide homology. The remarkable genetic homogeneity of these strains was stable with time, host species, and geographic distribution. Conversely, the S. Am. isolates displayed fingerprint patterns which differed markedly from the N. Am. strains and, in addition, exhibited significant genetic diversity amongst one another (oligonucleotide homologies 17-92%). Both geographic distribution and time appeared to influence the genetic relatedness of the S. Am. strains. Analysis of structural virus proteins supported these data. The N. Am. strains displayed a consistently similar protein migration pattern by PAGE, whereas more extensive protein variation was observed among the S. Am. strains. Although significant variations were demonstrated among N. and S. American EEE strains, the major antigenic determinants were conserved as demonstrated by Western blot analysis.

Our results suggest that a difference exists in selective pressures between N. and S. Am. EEE viruses, and points to the possibility that regional differences in vector populations and/or transmission cycles may play an integral role in EEE virus evolution.

361 YELLOW FEVER VIRUS EVOLUTION: COMPARATIVE ANALYSIS OF THE NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCES OF THE STRUCTURAL GENES OF TWO GEOGRAPHICALLY ISOLATED STRAINS.

*Mary E. Ballinger and Barry R. Miller, Division of Vector Borne Diseases, Center for Infectious Diseases, Centers for Disease Control, PHS, HHS, Fort Collins, Colorado 80522

Geographically distinct isolates of yellow fever virus (YFV) have been shown to vary in certain biological and physical characteristics. Specifically, studies have shown differences between South American and African YFV strains in their virulence for mice and in the physical properties of their envelope glycoprotein. Additionally, genetic heterogeneity has been demonstrated between such strains by oligonucleotide fingerprinting. In order to investigate the molecular basis for these variations, we have determined the nucleotide sequence of the structural genes of a Peruvian YFV isolate (1899/81) and compared it with the published (Hahn, et al.) sequence of the corresponding region of the African YFV Asibi strain. Additionally, a comparison was made of the deduced amino acid sequences of the two isolates. These YFV strains have evolved in separate ecological systems for presumably hundreds of years and have thus responded to different selection pressures imposed by environment, invertebrate vectors, and vertebrate hosts. As may be expected between RNA virus strains which have evolved in such varied ecosystems, we observed a great deal of heterogeneity at the nucleotide level. However, the deduced amino acid sequences of the two strains were remarkably homologous with only a few significant amino acid changes apparently responsible for the physical and biological variations observed.

N: MOLECULAR VIROLOGY

362 GENETIC RELATIONSHIP AMONG JAPANESE ENCEPHALITIS VIRUS STRAINS AS DETERMINED BY PRIMER-EXTENSION SEQUENCING

*Woan-Ru Chen, Rebeca Rico-Hesse and Robert B. Tesh. Yale Arbovirus Research Unit, Dept. Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

Primer extension sequencing of the RNA template of several viruses, such as polio and dengue, has provided new information on the geographic origin and evolution of these agents. In the present study, 45 strains of Japanese encephalitis (JE) virus from a variety of geographic areas in Asia were examined by this technique. Two hundred forty nucleotides from the C/pre-M gene region were selected for study, since this region showed more silent mutation than any other, and it provided sufficient information for determining genetic relationships among the virus isolates. Using 12% divergence as a cutoff point for virus relationships, the 45 isolates fell into 3 distinct genotypic groups. The maximum divergence across the C/pre-M gene region among the JE virus isolates was 15%. One genotypic group consisted of JE virus isolates from northern Thailand and Cambodia. A second group was comprised of isolates from southern Thailand, Malaysia and Indonesia. The rest of the isolates from Japan, China, Taiwan, Philippines, Sri Lanka, India and Nepal made up a third group. Strains in the second group came from regions where encephalitis is uncommon, suggesting they might be less virulent. Also of interest is the finding that the recently sequenced Nakayama strain is more like Indian strains of JEV than Japanese strains. Results of this study demonstrate that the comparison of short nucleotide sequences provides insight to JE virus evolution and transmission, although a different region of the flavivirus genome was studied versus dengue virus.

363 GENETIC ANALYSIS OF VARIATION IN THE NUCLEOTIDE SEQUENCES AND DEDUCED AMINO ACID SEQUENCES OF THE STRUCTURAL AND NS-1 NON-STRUCTURAL GENES FROM DENGUE 1 (16007) AND EARLY PRIMARY DOG KIDNEY PASSAGE STRAINS. *May C. Chu, Ravithat Putvatana and Dennis W. Trent. Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Ft. Collins, Colorado.

Dengue 1 (DEN-1) virus strain 16007 has been passaged in primary dog kidney (PDK) cells for development of an attenuated vaccine. At present, PDK passage 14 has been selected as the vaccine candidate and is currently under vaccine trials (Bhamrapravati *et al.* 1988). We have determined the partial envelope nucleotide sequence of DEN-1 16007 as part of our worldwide genetic analysis of DEN strains and have extended this to include the entire structural and nonstructural NS1 genes of 16007. To identify sequence changes that may be involved in the process of DEN-1 virus attenuation, we have begun to analyze some biological markers and the genome sequence of PDK passage 1. Virus plaque size in tissue culture and the ability of the virus to replicate in human macrophage cells indicate that virus plaque size and macrophage growth diminish between PDK passages 4 and 5. Determination of the nucleotide sequence of the parental, PDK-5 and PDK-8 passages show that sequences encoding the structural region, especially in the envelope change most (2%-8%). Examination of sequence changes between parental and PDK passages will enable us to understand molecular changes in the attenuated DEN-1 virus genome.

N: MOLECULAR VIROLOGY

EXPRESSION OF THE DENGUE-2 ENVELOPE GLYCOPROTEIN IN MAMMALIAN CELLS.

364 *E.P. KELLY, R.J. FEIGHNY, C.H. HOKE. Walter Reed Army Institute of Research, Washington, DC 20307-5100

The Dengue 2 Envelope glycoprotein (D2 E gp) is a major target of immune defense during virus infection. It is important to evaluate its role in immune protection for the development of a vaccine against virus infection. Since D2 virus grows to low titer in cultured cells, an alternative method, expression of the D2 E gp in mammalian cells, was selected for large scale production of the E gp for study. The D2 E gp gene, from strain PR 159, without the hydrophobic 3' coding region (1.4 Kb) was subcloned into a mammalian cell expression vector, pRSV.2, which contains the Rous Sarcoma Virus Long Terminal Repeat transcriptional promoter. The recombinant vector and a selectable cotransfecting plasmid were used to transfect Monkey Kidney (CV-1) cells. The presence of the D2 E gp gene was detected in transfected cells by southern blot hybridization of total cellular DNA with a labeled E gp gene probe. Cell lysates reacted with hyperimmune anti-Dengue 2 serum. Further characterization of this reactivity will be evaluated using monoclonal antibodies. Methods to extract and evaluate the structure and antigenicity of expressed D2 E gp are being developed.

RESPONSE IN MICE FOLLOWING INOCULATION WITH DENGUE-2 PROTEINS ISOLATED BY HIGH PRESSURE LIQUID CHROMATOGRAPHY.

365 *R.J. FEIGHNY, M.J. BURROUS, J.M. McCOWN, J.R. PUTNAK, C.H. HOKE.
Walter Reed Army Institute of Research, Washington, DC 20307-5100

Neither attenuated nor killed vaccines for dengue viruses have been successfully developed. Use of killed viruses is costly and may lead to immune dependant enhancement. The proper balance between attenuation and immunogenicity has been difficult to achieve. Synthetic peptide vaccines are of low antigenicity and have not yet led to the production of protective antibodies in mice. The development of recombinant vaccines necessitates purification of the protective antigen. In order to determine whether this is a viable alternative we have examined the use purified dengue-2 envelope glycoprotein as a vaccine tool. The advantages of using purified E are three-fold. First, there is no danger of reversion as in attenuated viruses. Second, use of HPLC technology on a large scale may reduce the cost per dose. Third, the size of the protein, about 60K, may lead to a greater antigenic response since the protein conformation will more closely represent the true configuration. We have purified the dengue-2 proteins by ion exchange HPLC to single bands on a SDS gel. These proteins have been injected into mice resulting in the production of antibodies against viral antigens in all animals as determined by western blotting of polyacrylamide gels.

N: MOLECULAR VIROLOGY

366 SYNTHETIC PEPTIDES DERIVED FROM THE E-GLYCOPROTEIN OF DENGUE 2 VIRUS DEFINE ANTIGENIC AND STRUCTURAL CHARACTERISTICS.

J.T. Roehrig*, A.R. Hunt, A.J. Johnson, R.A. Bolin and M.C. Chu. Centers for Disease Control, Ft. Collins, CO.

By computer modeling and analogy to results determined using Murray Valley encephalitis virus, we designed, synthesized and analyzed 18 synthetic peptides comprising 80% of the extramembranal region of the E-glycoprotein of dengue (DEN) 2 virus (Jamaica strain). These peptides were analyzed for reactivity with a variety of antiviral antibodies. Peptide binding was studied using hyperimmune mouse polyclonal and human immune sera from DEN1, DEN2 and DEN4 virus infections. The peptides were used to immunize BALB/c or outbred NIH-Swiss mice. Twelve of these peptides elicited anti-peptide antibody and ten of these elicited antiviral antibody. Linear peptides corresponding to amino acids 35-55, 121-140, 142-172, 225-249, 333-354 and 352-368 elicited highest titer antiviral antibody. Three non-linear peptides which predict "structural" regions based upon disulfide bonding and corresponding to sequences between amino acids 53 and 140 also elicited antiviral antibody. Only antibodies raised against two of these "structural" peptides showed increased reactivity with acid denatured virus. Data on the neutralization, hemagglutination inhibition, and protective capacities of these peptides will be discussed.

367 THE COMPLETE SEQUENCE OF VP5 OF BROADHAVEN VIRUS, A KEMEROVO SERO-GROUP ORBIVIRUS, AND ITS RELATIONSHIP TO BLUETONGUE VIRUS.

S.R. Moss and P.A. Nuttall. NERC Institute of Virology & Environmental Microbiology, Mansfield Road, Oxford, OX1 3SR, England.

The complete nucleotide sequence of VP5 from Broadhaven (BRD) virus, a Kemerovo serogroup orbivirus has been determined. It is coded for by genome segment 5 and is the major determinant of neutralization in the Great Island subgroup of Kemerovo viruses. It has been shown to be 1658 nucleotides in length, containing an open reading frame of 480 amino acids, capable of coding for a protein of 53K. The non-coding regions at the 5' and 3' ends are of 21 and 197 nucleotides in length respectively. On comparison with BTV10 segment 5, there was homology between the nucleic acids of 42% and 31% between the amino acids. This compares with 40% - 70% for the coat proteins of different serotypes of BTV. The conserved regions at the 5' and 3' ends were not identical to those of BTV. This data indicates that BRD and BTV are similar but not closely related. To investigate the relationship further, genome segments that may show greater homology to BTV are now being compared.

O: ENTOMOLOGY - LYME DISEASE

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NATIONAL SURVEILLANCE OF LYME DISEASE, 1987-1988

*T.F. Tsai, R.E. Bailey, G.W. Letson. Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins, CO.

In 1987 and 1988, 6958 Lyme disease cases from 43 states were reported to CDC. The 4,582 cases reported in 1988 was nearly double the number of cases reported in 1987, and ten-fold the number reported in 1982, when a systematic system of national surveillance was established. The average annual incidence of reported Lyme disease in the United States in 1987-1988 was 1.43/100,000. New York led the nation in reported cases in 1988 with 57% of the cases reported nationally. Eight states, New York, New Jersey, Pennsylvania, Connecticut, Massachusetts, Rhode Island, Wisconsin, and Minnesota, reported 92% of the nation's cases. Regionally, incidence rates were highest in Northeastern and Mid-Atlantic states, intermediate in North Central and Pacific states, lower in the Southeast, and lowest in the Great Plains and Mountains states. The seven remaining states in which Lyme disease has not been transmitted all lie west of the 100th meridian. Lyme disease cases were reported chiefly in children under 15 and in adults between 25 and 45 years of age. An outdoor occupation was reported in only 11% of cases. The proportion of cases in females, 52%, was higher than in previous reports.

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GROWTH KINETICS OF THE LYME DISEASE SPIROCHETE IN IXODES TICKS.

*J. Piesman, J.R. Oliver, and R.J. Sinsky. University of Alabama at Birmingham, Birmingham, AL.

Investigations, to date, on the interactions between the Lyme disease spirochete (Borrelia burgdorferi) and its vector tick (Ixodes dammini) have been largely descriptive in nature. We developed a simplified method for measuring the number of Lyme disease spirochetes within Ixodes ticks, and determined the abundance of spirochetes at different stages of the tick's life cycle. Groups of larval I. dammini, recently engorged on spirochete infected hamsters, were homogenized in PBS and the number of spirochetes contained in 5µl samples were counted using a direct fluorescent antibody probe. Spirochete abundance reached a maximum of 2,735 spirochetes per larval tick on Day 15 postrepletion. A 5-fold drop in spirochete levels occurred during the subsequent premolting period. Recently molted nymphs contained <300 spirochetes per tick. Following nymphal repletion, spirochete multiplication renewed, reaching densities of 61,275 spirochetes per nymph on Day 75 postrepletion. A 10-fold drop in spirochete abundance occurred when ticks molted to the adult stage, during Day 50-70. Those nymphs not molting to the adult stage, maintained high spirochete levels (ca. 50,000) from Day 75-135. Thus, spirochete growth was greatest in recently engorged ticks, and decreased dramatically in molting ticks. An understanding of the biochemical factors which control rapid growth of B. burgdorferi in Ixodes ticks could aid in the search to improve in vitro culture methods for the Lyme disease spirochete.

O: ENTOMOLOGY - LYME DISEASE

- 370 LYME DISEASE IN IOWA.
M.G. Novak, K.B. Platt, and W.A. Rowley. Iowa State University,
Ames, IA.

In 1988, at least 25 confirmed cases of Lyme disease occurred in Iowa. Many of these involved exposure within the state. The distribution of cases, largely throughout eastern Iowa, implies a range expansion of the principal vector, Ixodes dammini, from Wisconsin, a major focus of Lyme disease. Additionally, a reported secondary vector, Amblyomma americanum, inhabits much of southern Iowa. The presence of these two vector species, a rapidly increasing deer herd, and a rural demography, portend increased Lyme disease transmission in Iowa.

In 1989, a statewide Lyme disease program was initiated to study the biology of I. dammini and to determine the distribution and infection rate of this species in Iowa. Ticks were collected by a variety of methods including dragging, small mammal trapping, mail-in responses to notices posted at all county and state parks, and inspection of road-kill deer. All ticks collected were identified, and live I. dammini and A. americanum were tested (direct fluorescent antibody) for Borrelia burgdorferi spirochetes. Results from the initial year of these studies are summarized.

- 371 FACTORS STIMULATING IXODES-BORNE PATHOGENS TO MATURE.
Sam R. Telford III and Andrew Spielman.
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To determine whether tick-borne pathogens are stimulated to become infectious due to the heat or the food component of host contact, infected nymphal Ixodes dammini ticks were permitted to feed upon lizards (Lacerta viridis) continuously held at 15C, 24C, or 37C. In parallel, similarly infected, but nonfeeding ticks were held at these temperatures and infected ticks were placed on hamsters held at 24C. A piroplasm (Babesia microti) remained in the sporoblast stage of development within the salivary glands either of the lizard-feeding or the nonfeeding ticks held at 15C or 24C. Sporogony proceeded normally in lizard-feeding ticks held at 37C and in hamster-feeding ticks. Sporogony began but only rarely was completed in nonfeeding ticks held at 37C. A spirochete (Borrelia burgdorferi) remained confined to the lumen of the midgut of nonfeeding infected ticks regardless of ambient temperature. They disseminated to the hemolymph in hamster-feeding ticks or in those feeding on lizards, whether at 15C, 24C or 37C. These ticks fed to repletion on hosts incubated at any of these ambient temperatures, but engorged more rapidly as temperature increased. We conclude that no single stimulus induces the pathogens transmitted by this Ixodes tick to mature, but that heat and the presence of ingested food-material may act alone or in combination as proximal maturational stimuli.

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INVASIVENESS OF THE LYME DISEASE SPIROCHETE
UNRELATED TO HYALURONIDASE ACTIVITY.

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We determined whether a capacity to reduce the viscosity of hyaluronic acid (HA) may enhance the motility of the Lyme disease spirochete (*Borrelia burgdorferi*) and its capacity to traverse a membrane. Spirochetes were assayed for HAase activity by recording the viscosity of solutions of HA that had been incubated with spirochetes. In a complementary assay, extensive subcutaneous staining by Evans blue served as a criterion for HAase activity where spirochetes had been injected intradermally into rabbits. No evidence of such enzymatic activity was found. We then determined whether viscosity of the substrate affects motility of these organisms by correlating spirochete velocity with concentration of HA. Motility increased with viscosity and was greatest at viscosities in the range of extracellular matrix glycosaminoglycans. Indeed, a hyaluronidase would inhibit invasiveness by rendering these spirochetes relatively non-motile. The Lyme disease spirochete therefore, may differ from the pathogenic treponemes in that invasiveness appears to be independent of HAase activity.

373 LACK OF LYME DISEASE SPIROCHETE TRANSMISSION FROM RESERVOIR MICE
(*PEROMYSCUS LEUCOPUS*) TO THEIR OFFSPRING.

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White-footed mice (*Peromyscus leucopus*) serve as the primary natural reservoir for the Lyme disease spirochete (*Borrelia burgdorferi*). Vector ticks (*Ixodes dammini*) readily become infected after feeding on these reservoirs. In this study, we evaluated whether or not female mice, infected with this spirochete, transmitted infection directly to their offspring. To do this, mice were live-captured in several Massachusetts' sites where Lyme disease spirochetes are endemic. Pregnant females were held individually in cages in the laboratory through birth, and mothers and their offspring were caged together until weaning. Each female, and two offspring were then tested for their spirochete infectivity by infesting them with noninfected larval *I. dammini*. Tick-infested mice were held in wire mesh cages over water; upon detaching, all engorged ticks were collected and stored until examined for spirochetes as nymphs. All 12 of the mother mice examined infected ticks, and exhibited serum antibodies to *B. burgdorferi*. However, none of the 24 offspring tested produced infected ticks. Thus, it would appear that immature mice must first be infected by tick bite before becoming infective themselves.

P: AMEBIASIS

374 A MONOCLONAL ANTIBODY SPECIFIC FOR ELECTRON-DENSE GRANULES SECRETED BY Entamoeba histolytica

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A collagenase activity stimulated by collagen type I has been related with virulence of E. histolytica, thus suggesting its direct involvement in tissue invasion. Transmission electron microscopy has shown that this process is accompanied by formation and liberation of electron-dense granules (EDG). Consequently, we hypothesized a role for EDG in the pathogenesis of this parasite. To test this possibility, we purified and partially characterized EDG. We detected at least eight peaks of protein by FPLC and eight polypeptides by PAGE. The silver stain technique showed fifteen polypeptides among them were a 40KDa protein recognized by a monoclonal antibody (B-4) prepared against EDG. The polyclonal antibody recognized six polypeptides with molecular weights of: 18, 33, 47, 85, 92 and 103 KDa. B-4 resulted specific for EDG by ELISA and immunofluorescence. In addition, B-4 reacted with extracts from pathogenic E. histolytica trophozoites (strains: HM-1, HM-38 and HK-9) but not with those of non-pathogenic species such as E. moshkovskii and E. invadens or the protozoan Giardia lamblia. Consequently, we suggest that this antibody may be useful as a marker to make a differential diagnosis of enteric amoebiasis, and to study the biological role of EDG proteins. Supported by COSNET, SEP, México (441.85). Immunofluorescence studies were done in the "UNIDAD DE MICROSCOPIA ELECTRONICA" from CINVESTAV.

375 INHIBITION OF IN VITRO CYTOTOXICITY AND ION CHANNEL FORMING ACTIVITY OF ENTAMOEBAS HISTOLYTICA BY MURINE MONOCLONAL ANTIBODIES. *J.N. Aucott, I.J. Malholtra, R.A. Salata, Department of Medicine, Case Western

Reserve University and University Hospitals, Cleveland, Ohio.

Cytotoxicity leading to invasive disease due to Entamoeba histolytica (Eh) may be mediated by an ion channel forming activity which we have named poretoxin (PT). We prepared murine monoclonal antibodies (Mabs) to partially purified PT and examined their effects on Eh cytotoxicity and on PT-mediated Ca^{2+} flux in human polymorphonuclear neutrophils (PMN).

PT was prepared by triton-X-114 extraction of the particulate fraction of an ultracentrifuged axenic virulent HM1:IMSS Eh homogenate. Three Mabs, EH-C-7, EH-C-8, EH-C-9, were generated against PT as screened by ELISA. All were of the IgM isotype by immunodiffusion. Eh cytotoxicity was assayed by ^{51}Cr release from Chang liver cells. At a ratio of 100 Chang cells to 1 Eh, all 3 Mabs (10ug/ml) inhibited specific ^{51}Cr release by 44-91% at 5h when compared to an isotype-matched control Mab ($P \leq 0.01$).

The mechanism of inhibition of Eh cytotoxicity by the Mabs was examined using human PMN as model target cells. Mabs had no effect on adherence of Eh to PMN using a standard rosetting assay. Eh calcium channel forming activity was measured in PMN with the calcium-sensitive intracellular probe FURA II. Increased PMN intracellular calcium induced by PT (25ug/ml) was inhibited by all 3 Mabs (1:100 dilution) (56-87% inhibitions vs. control Mab, $P \leq 0.02$).

We have developed 3 Mabs against PT from virulent Eh all of which inhibit cytolysis of Chang liver cells and decrease PT-induced Ca^{2+} influx into human PMN. These Mabs will be used to purify PT and to further examine the role of this ion channel forming activity in the cytopathogenicity of E. histolytica.

P: AMEBIASIS

- 376 **CYTOKINE ACTIVATED HUMAN NEUTROPHILS KILL *ENTAMOEBA HISTOLYTICA* TROPHOZOITES IN VITRO.** K. Chadee* and M. Denis, Institute of Parasitology of McGill University, Ste-Anne-de-Bellevue, Qc, Canada H9X 1C0

Entamoeba histolytica (Eh) trophozoites contain a membrane-bound neutrophil (PMN) chemoattractant and upon direct contact with amebae PMN lysis occurs. The released neutrophil proteases lyse hepatocytes and have been implicated in forming the initial hepatic lesions in the pathogenesis of amebic liver abscess formation. In human and animal models of the disease, resistance to recurrent intestinal colitis or liver abscess occurs. While macrophages and lymphocytes have been shown to play a crucial role in mediating resistance to re-infection, the role of PMNs in immunoregulation in amebiasis is not known. We studied the capacity of recombinant interferon gamma (rIFN- γ) and tumor necrosis factor alpha (rTNF- α) to endow human PMNs with amebicidal activity. Unstimulated PMNs were rapidly killed by direct contact with virulent Eh (HMI-IMSS) trophozoites, whereas PMNs killed 100% of the non-virulent Eh-like Laredo amebae. Treatment of PMNs with rIFN- γ alone killed 30% of Eh trophozoites. However, rIFN- γ and rTNF- α pre-treatments in combination increased PMN killing to 70%. In the absence of direct contact between PMNs and amebae, rIFN- γ treated PMNs killed 70% of the amebae and rIFN- γ and rTNF- α pre-treatments in combination killed 97% of the parasites. PMN enhancement of amebicidal activity following cytokine treatments was correlated with increased PMN resistance to amebic contact-dependent killing and was shown to be 73% H₂O₂ dependent. The immunologic production of cytokines could, therefore, be important in the activation of PMNs for host defense mechanisms against amebiasis. (Supported by NSERC Canada).

- 377 **IDENTIFICATION OF THE FIBRONECTIN RECEPTOR OF *ENTAMOEBA HISTOLYTICA* AS THE 35 KDA SUBUNIT OF THE GAL/GALNAC ADHERENCE LECTIN.** B. J. Mann* and W.A. Petri, Jr. Department of Medicine, University of Virginia, Charlottesville, VA 22908

The galactose/N-acetyl D-galactosamine (Gal/GalNac) inhibitable lectin of *Entamoeba histolytica* has been shown to mediate amebic adherence to a variety of target cells including human colonic epithelial cells and colonic mucin. Monoclonal antibody affinity-purified Gal/GalNac lectin is a heterodimer composed of two glycoprotein subunits of 170 KDa and 35 KDa. Polyclonal antisera and monoclonal antibodies that recognize only the 170 kDa subunit have been shown to inhibit amebic adherence to target cells up to 100%, indicating that the 170 KDa subunit contains the galactose-binding cell adherence domain of the protein. The 35 KDa subunit is poorly immunogenic and no known function has been ascribed to it. Talamas-Rohana and Meza (J Cell Biol 106:1787-1794) have identified a 37 KDa amebic protein that binds fibronectin. The similarity in size and cell surface location of the fibronectin receptor and the 35 KDa subunit of the Gal/GalNac lectin prompted us to investigate if the 35 KDa subunit could bind fibronectin. Fibronectin labeled with ¹²⁵Iodine or biotin was used for these experiments. Fibronectin bound to a 35 KDa protein on western blots of total amebic proteins reduced with β -mercaptoethanol. Fibronectin bound to the 35 KDa subunit of the affinity-purified Gal/GalNac lectin reduced with β -mercaptoethanol on western blots. The binding of labeled fibronectin could be competed off with cold fibronectin, indicating specific binding of fibronectin to the 35 KDa subunit. We have demonstrated that the 35 KDa subunit can bind fibronectin and suggest that the 35 KDa subunit is the fibronectin receptor described for *E. histolytica*. This data suggests that the Gal/GalNac lectin represents a unique bifunctional protein with the ability to bind to the surface of intact cells in a galactose-inhibitable manner via the 170 KDa subunit, and to fibronectin, a component of the extracellular matrix via the 35 KDa subunit.

- 378 THE GALACTOSE AND N-ACETYL-D-GALACTOSAMINE (GAL/GALNAc) ADHERENCE LECTIN OF *ENTAMOEBIA HISTOLYTICA* IS DETECTED BY RIA ONLY IN PATHOGENIC STRAINS. William A. Petri, Jr*, Terry F.H.G. Jackson and David Mirelman. University of Virginia, Charlottesville, Virginia, Research Institute for Diseases in a Tropical Environment, Durban, South Africa, and Weizmann Institute, Rehovot, Israel.

Entamoeba histolytica infection results in either asymptomatic colonization or invasive colitis and liver abscess. *E. histolytica* isolates from patients with invasive disease have characteristic isoenzyme profiles (pathogenic zymodemes), suggesting a role for parasite factors in determining the severity of infection. The Gal/GalNAc lectin is a 260 kDa cell surface protein isolated from pathogenic zymodeme strain HM1:IMSS trophozoites. Inhibition of the lectin with Gal or GalNAc prevents amebic adherence to and contact dependent killing of target cells. We tested 48 pathogenic and nonpathogenic strains for the presence of the Gal/GalNAc lectin by radioimmunoassay (RIA). The RIA utilizes monoclonal antibodies directed against Gal/GalNAc lectin epitopes 1 and 3 and can detect as little as 1 ng of lectin. There was no measurable lectin in any of the 32 nonpathogenic zymodeme isolates tested. In contrast the lectin was uniformly present in all 16 pathogenic zymodeme isolates tested with the RIA, including isolates from Mexico, Thailand, South Africa, India, and the United States. Strains whose zymodemes had been changed by in vitro culture conditions (SAW 1734 and CDC 0784:4) only had detectable lectin when expressing a pathogenic zymodeme. In summary, the lectin has only been found by RIA in pathogenic zymodemes. The lectin RIA appears to be a simple and rapid method to distinguish pathogenic from nonpathogenic strains of amebae in culture. Further exploration of the reasons for the lack of monoclonal antibody reactivity to the Gal/GalNAc lectin in nonpathogenic strains may lead to a better understanding of the parasite virulence factors responsible for production of invasive amebiasis.

- 379 SERUM ANTI-ADHERENCE LECTIN ANTIBODIES AS A MARKER OF PATHOGENIC *ENTAMOEBIA HISTOLYTICA* INFECTION.

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Presently, detection of pathogenic *E. histolytica* infection requires expert microscopy, use of undefined crude parasite antigens in serologic assays, and starch gel electrophoresis to characterize the isoenzyme pattern (zymodeme). Previously, we found by immunoprecipitation of a limited number of immune sera that the amebic galactose-inhibitable adherence lectin is a major conserved antigen. We performed immunoblotting and ELISA using immunoaffinity purified adherence lectin to determine if serum anti-lectin antibodies are a marker of pathogenic *E. histolytica* infection. Sera were obtained from residents of South Africa determined to have amebic liver abscess (83), asymptomatic intestinal infection with pathogenic (4) or nonpathogenic zymodemes (65), and a negative stool culture for *E. histolytica* (32); for comparison, control sera were obtained from 40 healthy residents of Virginia and 29 North Americans infected with other parasites such as *Giardia*, *Cryptosporidium*, and non-*Entamoeba* amebae. By immunoblotting, 95 sera contained antibodies to total parasite protein; of these, 95% had serum antibodies to the 170 Kdalton heavy subunit of the adherence lectin. All 253 sera were studied using an adherence lectin-ELISA: anti-lectin antibodies were found in 99% of sera from liver abscess patients and 100% of sera from those with asymptomatic pathogenic infection. A negative ELISA was found in all sera from healthy American controls or those infected with other parasites ($p \leq 0.01$). South Africans having asymptomatic nonpathogenic infection or a negative stool culture demonstrated an equal 25% prevalence of serum anti-lectin antibodies, apparently due to prior pathogenic *E. histolytica* infection. In summary, immunoblotting confirmed that the adherence lectin is a conserved *E. histolytica* antigen; by ELISA we demonstrated that the presence of serum anti-lectin antibodies indicates current or past invasive amebiasis or asymptomatic infection with *E. histolytica* having a pathogenic zymodeme. This is the first report of a serologic test using a purified amebic antigen which has promise for use in diagnosis of pathogenic *E. histolytica* infection.

P: AMEBIASIS

- 380 ANTIGENICITY OF DEGLYCOSYLATED *ENTAMOEB* *HISTOLYTICA* PROTEINS.
*E.L.W. Kittler & J.I. Ravdin, University of Virginia School of Medicine, Charlottesville,
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In order to isolate the major conserved antigens of *E. histolytica* from a cDNA expression library in *E. coli*, which does not glycosylate proteins, it is necessary to develop probes specific for antigenic protein sequences rather than carbohydrate structures. Antibodies in pooled human immune sera (from patients cured of invasive amebiasis) recognize a conserved set of *E. histolytica* proteins which, as separated by SDS-PAGE, include 37, 43, 59, 90, and 110Kd molecular weight proteins, and the 170Kd heavy subunit of the galactose-inhibitable adherence lectin; all except the 43Kd protein are apparently glycosylated in *E. histolytica*. Isolated antigenic recombinant *E. histolytica* proteins can be used to determine conservation of Ig and T-cell recognition by immune individuals from different geographic regions; T cell epitopes may be more dependent on linear protein rather than carbohydrate structure. Oxidation of total amebic protein with sodium metaperiodate (NaIO₄) to remove carbohydrate alters the pattern of antigens recognized by immunoblotting with pooled human immune sera: the antigenicity of the 110Kd is conserved, 90 and 43Kd antigens are faintly recognized, new bands of 92 and 96 are evident, an increase in recognition of a 37Kd antigen is seen, no recognition of 90 and 59Kd proteins is present. These changes may have resulted from a shift in protein mobility on SDS-PAGE, loss of antigenic carbohydrate structures, or exposure of additional antigenic sites on deglycosylated proteins. Rabbit antiserum was produced by immunization with the deglycosylated *E. histolytica* protein; rabbit immune serum recognizes the periodate-treated amebic protein with 2000 fold greater sensitivity than the native protein. On Western blots of native and deglycosylated amebic protein the rabbit anti-serum recognizes some of the same antigens as human immune sera (native proteins of 37 and 43Kd; and deglycosylated proteins of 37, 43, and 92Kd) as well as additional antigens in both protein samples. Considering differences in antigenic recognition demonstrated by deglycosylation and producing protein-specific antibody probes are important strategies for the recovery of protein-specific antigens from an *E. histolytica* cDNA expression library.

- 381 IDENTIFICATION OF A SURFACE MOLECULE OF *Entamoeba histolytica* WITH IMMUNODOMINANT CHARACTERISTICS USING SERA FROM PATIENTS WITH HEPATIC ABSCESS. M.A. Meraz*, U. Edman, N. Agabian and I. Meza. CINVESTAV-IPN. and School of Pharmacy, U. of California San Francisco.

The capacity of invasive amebas to damage cells and penetrate solid organs is considered to be a multifactorial process requiring as a first step, surface contact between trophozoites and targets. The study of amebic surface antigens, their role in adherence, cell lysis and invasion of tissues, and their potential to trigger the immune humoral and cellular responses of the host, is then of great importance to understand pathogenicity, and also for future development of vaccines or diagnosis probes. 108 sera from patients with hepatic abscess were used to identify *E. histolytica* proteins that could be surface antigens and immunodominant in character. More than 60% of the sera recognized 8 peptides of 220, 190, 160, 130, 95, 75, 60 and 46 KDa. The 160, 95 and 46 KDa peptides were recognized by almost 90% of them. The sera precipitated *E. histolytica* mRNA in vitro translation products of MW in the range of the peptides identified as immunodominant so, the sera were utilized to isolate positive clones from an *E. histolytica* cDNA library made in λ gt11. 46 positive clones were isolated. Six of these clones were recognized by 13 out of 29 individual sera. The M-17 clone reacted with 90% of the individual sera, and expressed a fusion peptide identified by immunoblot using a pool of sera from patients with hepatic abscess and antibodies against amebic plasma membrane. Clone M-17 has an insert of 1.9 Kbp that by northern hybridization recognized a mRNA band of 3 Kb big enough to codify for the immunodominant peptide of 95 KDa. Affinity purified antibody against the M-17 fusion peptide specifically recognized the 95 KDa band in immunoblots of amebic cell extracts, and in live trophozoites induced the formation of Ab/Ag complexes, indicating its location on the amebic surface. Southern blot analysis of *E. histolytica* DNA cut with EcoRI, HindIII, BglII, and EcoRV showed a single band of different size when using the M-17 probe. The M-17 peptide is then codified by a single gene present in pathogenic *E. histolytica* such as HM1-IMSS, HM3-IMSS, HM38-IMSS and HK9. Corresponding DNA sequences were not found in other *Entamoeba* species or other protozoan parasites tested. The specificity of this probe could render it useful for diagnosis. On the other hand, the immunodominant character of this peptide could be valuable in studies of immunoprotection.

Q: TAPEWORMS

382 EVALUATION OF A HYDATID DISEASE CONTROL PROGRAM IN THE XINJIANG/UYGUR AUTONOMOUS REGION, PRC (CHINA).

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A program for hydatid disease control was evaluated in 16 randomly selected villages in 2 communities in Hutubi County (Xinjiang/Uygur Autonomous Region), an area in which hydatid disease is known to be endemic. Factors tested included the effect of: 1) the role of a village hydatid disease control officer ["CO"], 2) the use of praziquantel-medicated "bait" tablets for treatment of *Echinococcus granulosus* tapeworms in dogs, and 3) the use of educational materials for children and adults. Four villages comprising Group I ("controls") had no CO's, no drug for dogs, and no educational materials. The 4 villages in each of the other 3 groups had contact from a CO and received their supply of medicated tablets and educational materials at the first of the trial year only (Group II), on a monthly basis (Group III), or twice each month (Group IV), respectively. Evaluation of the contrasting treatment levels was done by determining the dog infection levels in 30-40 randomly selected dogs from each of the 16 villages before and after the trial period, and by use of pre- and post-treatment questionnaires distributed to 40 randomly selected households in each village. Infection levels in dogs during the 1-year period decreased in all treatment groups including the control villages (an average decrease from 16.2% to 4.3%), but decreased most (14.4% to 0.9%) in group IV. A statistically significant increase in knowledge levels and a change towards favorable behavior modification was noted most in Group III.

383 COUNTERIMMUNOELECTROPHORESIS USING AN ARC 5 ANTIGEN IS SPECIFIC FOR DIAGNOSIS OF CYSTIC HYDATID DISEASE. P.R. Hira*, H. Shweiki, I. Francis., K. Behbehani. Fac. of Med., Kuwait Univ., P.O. Box 24923, Safat, 13110 Kuwait.

A rapid and simple counterimmunoelectrophoresis (CIEP) on a cellulose acetate membrane using antigen that elicits an arc 5 precipitin line in IEP is sensitive for the diagnosis of cystic hydatid disease (CHD). We further determined the specificity by assaying sera of patients (i) infected with a variety of parasitic infections reported to elicit a non-specific reaction due to cross-reacting antigens (ii) with other comorbid conditions that present a problem in the differential diagnosis of CHD. We tested sera of patients with amoebiasis, schistosomiasis, *Taenia solium* cysticercosis, filariasis, and infections due to a variety of soil-transmitted nematodes. Other sera analysed were of patients with autoimmune disorders, malignancies reported to cross-react with hydatid antigens and conditions in which a space-occupying mass or lesion was presumed to be a hydatid cyst on ultrasound (US) and/or computerized tomography (CT). Compared to the indirect hemagglutination (IHA) test, the CIEP was 100% specific. Thus far, of over 1000 sera assayed of patients with a variety of medical conditions we found only a single false-positive. We conclude that the CIEP is ideal because of specificity of the antigen moiety, for laboratory diagnosis and also possibly for seroepidemiological studies.

Q: TAPEWORMS

- 384 ALBENDAZOLE THERAPY OF ECHINOCOCCUS MULTILOCULARIS INFECTION IN THE MONGOLIAN JIRD (MERIONES UNGUICULATUS). P.M. Schantz, F.H. Brandt, C. M. Dickinson, C.R. Allen, J. M. Roberts and M.L. Eberhard. Parasitic Diseases Branch, Center for Infectious Diseases, CDC, Atlanta.

We used an animal model to determine the response of Echinococcus multilocularis to albendazole administered at various dose levels and treatment schedules. Female weanling jirds (Meriones unguiculatus) were infected by intraperitoneal inoculation of protoscolices. Beginning 30 days postinfection, groups of 15 infected animals were fed 0.05% albendazole medicated feed (ABZ-MF) for one, two or three periods of 28 days separated by 14-day intervals. Mortality during the course of infection was reduced in all treatment groups, most strikingly in Group 5 that received 3 courses (6%) as compared with the nonmedicated control group (83%). Frequency of metastatic extension and mean metacestode weights were significantly less in animals of Group 5 than in controls ($p < .001$). The microscopic appearance of metacestode tissue in all animals of Group 5 indicated extensive necrosis, however, metacestode tissue from all treated animals survived and grew when inoculated into clean jirds. In a second experiment 3 groups of infected jirds received ABZ-MF at 0.01%, 0.05% or 0.10% for 150 days. There was a strong inverse correlation between final metacestode weights and serum concentrations of albendazole metabolites. Albendazole significantly inhibited metacestode growth, improved survival of infected jirds and conferred other clinical benefits, however, even with serum drug levels similar to those reported in treated humans, therapy failed to kill E. multilocularis.

- Cysticercosis Surveillance - Los Angeles County
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385 Los Angeles County Department of Health Services and Centers for Disease Control, Atlanta
GA

Cysticercosis surveillance was initiated in Los Angeles County in January, 1988 to more accurately assess the incidence of cysticercosis, determine the level of locally acquired and travel-associated cases and evaluate household members as possible sources of infection. Through April, 1989 80 (1/100,000) cases of confirmed or suspected cysticercosis were identified; 6 (7.5%) cases were fatal. The most common sites of infection were cerebral (84%) and ocular (7%). Eighty-seven percent of cases were immigrants who had presumed exposure in their country of origin. Latino's accounted for 75 (95%) of reported cases and rates (2.6/100,000) were highest in this group. The female-to-male ratio was 1.5:1; the mean age was 29.2 years (range 1-81). Five (6.3%) probable travel-associated cases, one of them fatal, occurred among residents who were born in the United States and had traveled to Mexico. Four (5.0%) autochthonous cases were documented. For one of these cases 2 close personal contacts were found to be infected with an adult Taenia presumably T. solium and represented the likely source of infection. In contrast, assessment of contacts for 27 cases whose likely source of infection was abroad found no tapeworm carriers. ($P = .03$) Cysticercosis causes significant morbidity and mortality among residents of Los Angeles County principally among Latino immigrants. However both locally acquired and travel-associated cases occur. Screening household and other close personal contacts of autochthonous cysticercosis cases can identify tapeworm carriers who can be treated and removed as sources of infection.

Q: TAPEWORMS

- 386 The Examination of the Tongue, a Rapid Method for the Diagnosis of Porcine Cysticercosis compared to serology using the ELISA or Immunoblot assay.

Emico Armando Gonzalez, Vita Cama, Robert H. Gilman, V. Tsang, J. Brandt, Elba Miranda, T. Montenegro and M. Verastigui
Universidad de San Marcos, PRISMA, Universidad de Cayetano Heredia, John Hopkins University and CDC.

Swine cysticercosis is part of the *Taenia solium* life cycle (1-3), a severe zoonotic disease. It causes major economic losses to pig husbandry. Throughout South America, farmers make the diagnosis of cysticercosis by examining the tongues of their pigs for the presence of cysticercotic nodules. Pigs thought to have cysticercosis are not brought to the slaughter house for fear of confiscation. Therefore reliable statistics on porcine cysticercosis only can be acquired using a method that can be used at the household level.

We compared results of the tongue test and two serological methods for the detection of cysticercosis, the ELISA and the Enzyme-Linked Immuno-electrotransfer Blot Assay (EITB) with results obtained by necropsy. We examined 18 tongue positive animals, 69 tongue negative animals from an endemic zone and 32 tongue negative animals from a zone free of cysticercosis. The Tongue test has a sensitivity of 0.72 and a specificity of 0.98, EITB a sensitivity of 0.96 and a specificity of 0.82, and the ELISA test a sensibility of 0.80 and a specificity of 0.62. The tongue examination is a relatively sensitive, highly specific test that can be used for village survey data.

- 387 Sero-epidemiology of Cysticercosis of a Rural village in Peru Using the Immunoblot Assay

The Cysticercosis Working Group in Peru and the CDC. Presented by R. Gilman. UPCH, Lima, Peru

We sampled a rural, high jungle Peruvian village using the new highly sensitive and specific immunoblot assay. Eighty percent of the houses of the village had pigs which roamed freely. Sera from 359 inhabitants of the 452 inhabitants (79%) were obtained and sampled by immunoblot. Stools were collected from 305/452 (67%) of the population. The stools were examined directly and after concentration for the presence of *Taenia sp.* eggs. In addition, the tongues and sera of all pigs over the age of 3 months were examined in order to determine what percent of the pigs had evidence of cysticercosis.

The results demonstrated a high prevalence of cysticercosis antibodies in the population 30/359 (8%). When children under 10 years of age were excluded the rate of seropositivity was 27/209 (13%). The youngest blot positive child was 8 years old. Of the 25 cases that were positive and that had a stool examination only 1 (4%) had *Taenia sp.* present in his stool. Seropositive cases were spread throughout the village without evidence of clustering. Two of the 27 families had more than one individual positive.

Infection with cysticercosis is frequent in endemic areas of Peru.

Q: TAPEWORMS

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Serological Diagnosis of Cysticercosis - Comparison of the ELISA assay for antibody (EAB) and antigen detection (EAG) compared to the Enzyme Immuno-transfer Blot (EITB) test.

The Cysticercosis Working Group in Peru and the CDC. Presented by F. Diaz. UPCH, Lima, Peru.

Patients with cysticercosis account for more than 10% of patients admitted to neurological hospitals in Mexico and Peru. Diagnosis is difficult because of the protean manifestations of the disease. Diagnosis by serology is useful but until recently has been insensitive. We have compared three serological methods for their diagnostic efficacy in patients with tissue-confirmed cysticercosis. The following three assays were used: the ELISA using crude antigen for the detection of antibody (EAB); the ELISA using a rabbit polyvalent antiserum to crude antigen, for the detection of antigen in spinal fluid (EAG); and the EITB assay using purified cysticercosis glycoprotein antigens. There were 29 sera and 19 CSF specimens available from 30 patients with tissue confirmed cysticercosis. In sera, 28 were diagnosed using EITB versus 23 by EAB. In CSF, 19, 15, and 12 patients were diagnosed by EITB, EAB, and EAG respectively. In order to evaluate specificity, sera from 51 subjects from Bangladesh, a non-endemic zone, were tested. None were positive by EITB while 19 had false positive results by EAB. The EITB is a highly sensitive and specific test for the diagnosis of cysticercosis.

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IMMUNOBLOT ASSAY OF ANTIBODY RESPONSES IN PIGS WITH CYSTICERCOSIS FROM NATURAL INFECTIONS AND MODULATED ISOTYPIC ACTIVITIES IN EXPERIMENTAL ANIMALS.

*J.C.W. Tsang, J.A. Brand, W. Zhou, A.E. Boyer, E.I.P. Kammago Solle, M. Rhoads, D. Murrell, P.M. Schantz, and R.H. Gilman. Centers for Disease Control, Atlanta, GA 30333, Agricultural Research Service, Beltsville, MD 20705, and U. Peruana Cayetano Heredia, Lima, Peru.

We reported the development and the result from laboratory trials of an immunoblot (EITB) assay for human cysticercosis (Tsang, et al, J. I. Dis. 159:50, 1989). This diagnostic assay is 98% sensitive and 100% specific. If proven functional in the pig, this test can be an important adjunct to epidemiology. We applied this assay to 4 pigs, experimentally infected with *Taenia solium* eggs derived from a human. Antigen-specific activities were observed as early as 1 week postinfection. The first antigen-specific isotypic response was IgM antibodies directed against a glycoprotein at 97 kD (GP97). This activity generally disappeared between the 6th and 9th week postinfection. Between weeks 5 and 8, IgG activity rose as IgM activity fell. The IgG activity, however, was directed mostly towards GP50 and GP42 antigens. If the same response occurs in people with cysticercosis, identifying specific isotypic activity may help to distinguish new infection from old. This assay was also applied to naturally infected pigs from Peru and U.S. domestic pigs with heterologous infections. The results of test efficiency for pigs will be reported.

Q: TAPEWORMS

390 EFFECT OF PRAZIQUANTEL ON TAENIA SOLIUM CYSTICERCI IN VITRO.

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The effect of Praziquantel on some physiological parameters of Taenia solium cysticerci was evaluated. Parasites were incubated in vitro with the drug and the following parameters were evaluated: oxygen consumption, evagination, protein release and morphological damage. The oxygen consumption rate was significantly lowered with 3.2×10^{-6} M, of Praziquantel. Evagination was totally inhibited with 3.2×10^{-4} M to 3.2×10^{-6} M, while 50% inhibition was attained with 3.2×10^{-8} M and 3.2×10^{-9} M. Praziquantel at 3.2×10^{-4} M and 3.2×10^{-5} M increased protein release from the parasites; furthermore cysticerci incubated with these concentrations showed tegumental damage and lose of tegument in some areas. Evaginated cysticerci incubated with all concentrations of Praziquantel showed rostellar protrusion; the degree of neck contraction was variable: high doses induced tetanic contraction while 3.2×10^{-9} M induced a significantly relaxation of the neck (defined by an increase in the size of the worm). These data suggest that Praziquantel probably has different effects on cysticerci's muscle cells: high doses may increase the intracellular calcium level, while low doses probably cause calcium release.

391 REPETITIVE DNA SEQUENCES IN TAENIA SOLIUM AND TAENIA SAGINATA.

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Neurocysticercosis is a human disease caused by the larvae of the tapeworm Taenia solium. Neurocysticercosis is acquired by ingestion of T. solium eggs, produced by the adult tapeworm living exclusively in the intestine of humans. However, a closely related species, T. saginata, whose larvae is not infective to humans, is also found in human intestine. Coproparasitoscopical techniques can not differentiate T. solium from T. saginata eggs. To ascertain if specificity could be obtained when comparing DNA from T. saginata with T. solium, eggs and genomic DNA were obtained and dotted onto nitrocellulose membranes. T. saginata DNA, used as probe, hybridized only slightly with T. solium adult worm DNA or with eggs. Unexpectedly, T. solium DNA used as probe, hybridized with T. saginata eggs even better than with homologous egg samples and similar hybridization was obtained with homologous and heterologous adult worm DNA.

A reasonable explanation is that T. saginata and T. solium share DNA sequences, present in a higher number of copies in the first species. The presence of repetitive sequences was tested by restriction enzyme analysis of DNA samples from both species. One to three repetitive fragments ranging from about 2 to more the 10 Kb were detected in T. saginata DNA digested with 5 out of the 7 enzymes tested. In contrast, only one repetitive fragment was observed when T. solium DNA was digested with one of the seven enzymes. These results are consistent with a higher number of repetitive sequences in T. saginata as compared to T. solium.

R: FILARIASIS - ONCHOCERCIASIS AND IVERMECTIN

- 392 IMPROVED METHOD FOR EXAMINING THE STRUCTURE AND REPRODUCTIVE STATUS OF ONCHOCERCA VOLVULUS.
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This method permits a sequential histological study of O. volvulus worms along their whole body length, thus avoiding some disadvantages of nodule sections and embryograms. Nodules fixed in ethanol/glycerol are digested in collagenase. The worms extracted are stained in Mayer's haematoxylin and brought into glycerol for clearing and untangling. Starting at the tail, each 5 cm "beat" is cut off as it is freed from the worm ball. The "beats," mounted on slides in glycerol, provide longitudinal views of all organs and embryonic stages. Each "beat" is then cut into 0.5cm lengths, which are doubled on themselves into a U-shape and implanted into slits cut in a 1.5 x 1.0 x 0.5 cm block of formalin-fixed, veal brain white matter, also in glycerol. The brain block is processed for histology giving transverse sections (4 per cm) all along the worm.

Female worms shed oocytes into the seminal vesicles continuously, although their numbers decrease in old age. In the seminal vesicles the oocytes undergo meiosis, each producing one ovum and 2 polar bodies. If the worm is (re-)inseminated one sperm penetrates each oocyte and later fuses with the ovum to form the zygote, which then develops to a microfilaria. When no sperm is present, the nucleus of each ovum rounds off and disappears and the ova degenerate to an amorphous, eosinophilic mass, as they pass down the uteri. (Re-)insemination will (re-)start embryonic development in a non-productive female; exhaustion of the sperm supply will cause the worm to revert to the non-productive state; and a few worms are found changing from one state to the other. No evidence is seen of a regular cycle of reproductive activity.

The method also reveals the detailed effects of ivermectin and other filaricides on the worm's reproductive system.

- 393 INITIATION OF VERTEBRATE-PHASE DEVELOPMENT BY LARVAL FILARIAE: ROLES OF TEMPERATURE AND MAMMALIAN SERUM IN DEVELOPMENTAL ACTIVATION.

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Upon passage from the vector to the vertebrate host, third-stage filarial larvae (L3) undergo abrupt changes in their physicochemical environments. The contributions of changing parameters to initiation of vertebrate-phase development, either as stimuli or as general growth requirements, remain unclear. The present study sought to define the roles of two factors, temperature and vertebrate serum, in the molt cycles of filariae from two genera, Dirofilaria and Onchocerca in an in vitro system. Percent molting in both species was directly proportional to the concentration of fetal bovine serum (FBS). Maximum molting occurred at concentrations of FBS as low as 5% for D. immitis and 8% for O. lienalis. Transfer of larvae from medium with 20% FBS to serum-free conditions after various periods of cultivation indicated that maximal molting occurred in D. immitis after exposure to FBS for only the first 16 hours in culture. In O. lienalis a minimum of 40 hours' exposure was required for maximal molting. Completion of the L3-L4 molt cycle was also temperature dependent in D. immitis with the minimum requirement being 30°C, the optimum 37°C, and complete inhibition occurring at 40°C. Cultivation for various periods at 37°C prior to transfer to the non-permissive temperature of 27°C indicated the temporal requirements for elevated temperature. Molting frequency increased sharply among larvae exposed to 37°C for between 24 and 36 hours, and larvae exposed for 48 hours exhibited a maximal molt response subsequently. These results suggest that temperature and serum act as early stimuli or triggers rather than as general requirements for the L3-L4 molt cycle. Supported by the UNDP/World Bank/WHO and the E. M. Clark Foundation.

R: FILARIASIS - ONCHOCERCIASIS AND IVERMECTIN

- 394 EXPERIMENTAL ONCHOCERCA VOLVULUS INFECTION IN A MANGABEY MONKEY (CERCOCEBUS ATYS): PARASITOLOGICAL AND IMMUNOLOGICAL OBSERVATIONS.
Mark Eberhard,* Anne Boyer, Jennifer Dickerson, Victor Tsang, Frank Richards, Rodolpho Zea-Flores, Essie Walker, and Guillermo Zea-Flores. Division of Parasitic Diseases, CDC, Atlanta, Georgia; Yerkes Primate Center, Emory University, Atlanta, Georgia; and Ministry of Health, Guatemala.

Onchocerca volvulus has been one of the most difficult human filariae to study because its strict host specificity has hindered the establishment of a suitable laboratory host. Chimpanzees have been proven to be good hosts for the parasite, but their availability and cost has limited their use in experimental studies. We were interested in establishing O. volvulus in a small primate species as an alternative model for study. Two species of primate were chosen for study; patas monkeys (Erythrocebus patas) and mangabey monkeys (Cercocebus atys). Three chimpanzees, 10 patas and 3 mangabey monkeys were inoculated with L₃ of Guatemalan origin. All animals were bled biweekly, and commencing at 8 months postinoculation skin snips were taken. One mangabey (inoculated with 43 L₃) and one chimpanzee (100 L₃ initial inocula + 330 L₃ in 4 subsequent inocula) developed antibody activity to a group of low molecular weight (<14,000 KD) antigens from adult O. volvulus extracts at 9 and 12 months, respectively. At 16 months postinoculation, the mangabey became skin snip positive, as did the chimpanzee at 21 months postinoculation. The appearance of antibody activity 7 to 12 months prior to the first detection of microfilaria in the skin may reflect commencement of mating activity, or release of reproductive products by male or female worms. We are currently evaluating the potential of mangabey monkeys to serve as models for onchocerciasis and comparing the immunological response of primates to that of man. (Supported by E. M. Clark Foundation and NIH grant RR00165).

- 395 ISOLATION AND CHARACTERIZATION OF EXPRESSION cDNA CLONES ENCODING ANTIGENS OF ONCHOCERCA VOLVULUS. S. Lustigman and A.M. Prince. The Lindsley F. Kimball Research Institute of The New York Blood Center, New York, NY.

To facilitate biochemical studies of potentially protective filarial antigens, a cDNA library constructed from adult O. volvulus (provided by Dr. J.E. Donelson) was screened with sera from chimpanzees immunized with X-irradiated third stage larvae (L3's). The sera recognized a limited set of L3 filarial antigens of approximately 20, 24, 40, 44, 60 and 88 KDa by Western blotting. Five clones (OV2, OV7, OV8, OV103 and OV106) were identified, sequenced and further characterized. OV2 (900 bp) and OV8 (1200 bp) are sibling cDNA clones that are not recognized by sera taken from infected chimpanzees or infected humans. Clones OV7, OV103 and OV106 are reactive with these sera. Clone OV103 is strongly reactive with serum from an infected chimpanzee which was amicrofilaremic. Clone OV7 is the only one that is recognized by rabbit serum raised against L3's of O. lienalis. None of the clones are cross reactive with sera from individuals infected with Wuchereria bancrofti. Monospecific affinity purified chimpanzee or human antibodies reactive with recombinant antigen produced by the OV7, OV103 and OV106 clones identified on Western blots the antigens of 17 KDa, 26-30 KDa and 14 KDa, respectively. The purified fusion polypeptides of these clones may be used to study the immune response to the infectious form of the parasite.

R: FILARIASIS - ONCHOCERCIASIS AND IVERMECTIN

- 396 PRIMARY STRUCTURE AND SUBCELLULAR LOCALIZATION OF AN *ONCHOCERCA VOLVULUS* LOW MOLECULAR WEIGHT ANTIGEN. E. Lobos*, N. Weiss and T.B. Nutman. Laboratory of Parasitic Diseases, NIH, Bethesda, MD and Swiss Tropical Institute, Basel Switzerland.

The primary structure of a species-specific immunodominant antigen of the filarial parasite *Onchocerca volvulus* was deduced from cDNA sequence analysis. Using affinity-purified antibodies from patients with onchocerciasis from West-Africa, we have isolated a cDNA clone from a λ gt11 cDNA expression library derived from microfilariae-producing female *O. volvulus*. The open reading frame encodes 152 amino acids, and the deduced sequence predicts a M_r of 16.85 kDa containing a putative signal peptide of 16 amino acids, likely to be important for protein transport. From Northern analysis, the mRNA coding for this antigen was estimated to have a size of 950 nucleotides. Immunoelectron microscopy established that the antigen encoded by this clone is present in the hypodermis, the cuticle, and the uterus of the filarial worms. Analysis of the T and B cell epitopes of the 16.85 kDa antigen is currently in progress using synthetic peptides. Since this antigen appears to be recognized exclusively by sera of patients with onchocerciasis and not by sera from patients with *Wuchereria bancrofti*, *Loa loa* and *Mansonella perstans*, it may prove to be an especially valuable tool for improving the specific diagnosis of onchocerciasis. Interestingly, a preliminary study of sera from children in a hyperendemic savanna area, indicates that antibodies against this antigen become detectable even in low-level patent infections.

- 397 IDENTIFICATION OF A FILARIAL ANTIGEN IN SERA FROM PATIENTS WITH ONCHOCERCIASIS. R. Chandrashekar*, A.F. Ogunrinade, O.O. Kale, and G.J. Weil. Washington University, St. Louis, MO and University of Ibadan, Ibadan, Nigeria.

The purpose of this study was to identify and characterize parasite antigens in sera from humans infected with *Onchocerca volvulus*. Immune complexes were precipitated from human sera with polyethylene glycol and analyzed by immunoblot with rabbit antibodies to *O. volvulus*. One parasite antigen was identified in *O. volvulus* sera with an apparent M_r of 23 kDa. Preliminary characterization studies indicate that this antigen is susceptible to degradation by Pronase but resistant to trypsin and to periodate oxidation. In addition, it does not contain phosphorylcholine. The 23 kDa antigen was detected in 16 of 18 sera from Nigerian onchocerciasis patients and in 4 of 9 endemic control sera. However, it was not present in nonendemic control sera from Nigeria or the U.S., including sera from patients with autoimmune diseases. We hope that these studies will lead to improved methods for diagnosis and quantitation of *O. volvulus* infection based on antigen detection.

R: FILARIASIS - ONCHOCERCIASIS AND IVERMECTIN

398 SPECIFIC AND CROSS-REACTING ANTIBODIES IN HUMAN RESPONSES TO ONCHOCERCA VOLVULUS AND DRACUNCULUS MEDINENSIS.

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Immunoelectroblotting and ELISA were used to identify non-cross reacting components of *Dracunculus medinensis* and *Onchocerca volvulus*, *Loa loa*, *Wuchereria bancrofti*, *Brugia malayi* and *Mansonella ozzardi*. Specific serodiagnostic ELISA systems for onchocerciasis and dracunculiasis were devised. PBS extracts of adult worms were depleted of phosphorylcholine (PC). Crude and PC-depleted extracts were reacted by ELISA with individual sera from subjects infected with a range of nematodes. Binding of total Ig, IgG, or IgG4 was studied. Crude extracts were cross reactive but specificity of ELISA was improved by restricting reaction to host's IgG4 antibody subclass, and/or by removing PC determinants from crude antigens. Parallel immunoelectroblots of crude and PC-depleted extracts were probed with pooled sera and developed to reveal binding of total Ig or only IgG4. Extensive cross recognition occurred, but useful diagnostic antigens were noted: a 12 kDa protein from *D. medinensis* and 14, 18, and 27 kDa proteins from *O. volvulus*. Two *Onchocerca*-specific ELISA systems non-reactive with antibodies to *D. medinensis*, were devised: a direct system using an isolated, surface-derived antigen and an inhibition assay using monoclonal IgG1 antibody directed against 15.4 and 29.5 kDa proteins. A *Dracunculus*-detecting ELISA of enhanced specificity was also developed using PC-depleted antigen and mouse antihuman-IgG4 to measure subclass-specific binding.

399 IVERMECTIN UPTAKE AND DISTRIBUTION IN THE PLASMA AND TISSUES OF SUDANESE AND MEXICAN PATIENTS INFECTED WITH *ONCHOCERCA VOLVULUS*

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Ten Sudanese patients with *Onchocerca volvulus* infection each received a single oral dose of 150 ug/kg of ivermectin. Plasma samples were collected at time 0, 1/2 hr, 1 hr, 3 hr, 4 hr, 6 hr, 12 hr, day 1, day 2, day 3, day 7, and day 30. Five patients were selected for nodulectomy and skin biopsies at 6 hr, 18 hr and 3 days post treatment. *O. volvulus* worm fragments were dissected free of host nodular tissues for ivermectin extraction. Plasma samples were collected from 22 ivermectin treated Mexican onchocerciasis patients, only at time 0, and 4 hr post treatment. To determine ivermectin concentrations a radioimmunoassay was developed and validated, and results were compared with HPLC. Plasma samples (50 ul) were used without extraction, but for tissues 100 mg samples were extracted using acetone and acetonitrile. IVM was detected in the plasma at 1 hr, peak concentrations were reached in an average of 5.6 hr. and the drug persisted at detectable levels through day 7. Ivermectin was present in the nodular tissue at 6 hrs and persisted for 3 days. IVM was detected in a separate worm tissue extract at a concentration similar to the nodule, but in subcutaneous fascial tissue higher amounts were found. Microfilarodermia declined quickly in all of the Sudanese and all but two of the Mexicans. Plasma concentrations in the two non-responders showed adequate absorption of IVM at 4 hours. Further studies with this technique may permit assembly of a more complete picture of the disposition of ivermectin in host and parasite tissues, and its relationship to efficacy.

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MASS TREATMENT OF ONCHOCERCA VOLVULUS INFECTION WITH IVERMECTIN. BM Greene, MC Pacqué, B Munoz, Z Dukuly, A Nara, C Elmets, AT White, HR Taylor, Dana Center, Wilmer Institute, Johns Hopkins University, Baltimore, MD, Divisions of Geographic Medicine and Cardiology, Department of Medicine, and Department of Dermatology, Case Western Reserve University, Cleveland, OH, and Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

Ivermectin is an effective drug for the treatment of onchocerciasis. Assessment of its safety during community-based treatment was undertaken to ascertain its acceptance and discern any rare adverse effects. We treated the population of a rubber plantation (14,000 people) in Liberia, where over 90% of the adults have *O. volvulus* infection. In 1987, 7,700 eligible people were treated; 101 had moderate adverse reactions. Following re-treatment of the population in 1988, only 37 people had adverse reactions. Of these, 21 were treated for the first time in 1988. Of the 101 people with adverse reactions during the first treatment, 5 refused re-treatment and 62 were retreated in 1988; 49 had no reaction and 13 had only mild reactions. No serious birth defects were found and no deaths occurred that could be related to ivermectin treatment. A sub-study of 32 older men (mean age 61) with high risk for organic heart disease was undertaken. Of these, 62% had baseline electrocardiographic abnormalities, including non-specific intraventricular conduction abnormalities (16), left anterior hemiblock (6), poor R wave progression (7), first degree AV block (7), supraventricular premature beats (2), and left axis deviation (7). Early repolarization was seen in 5. EKG tracings done every twelve hours after ivermectin administration showed no significant abnormalities in any of the subjects, and there were no significant changes in blood pressure or pulse. A further study of twenty one separate individuals with reactive onchodermatitis was undertaken. Skin lesions were photographed before and three months after treatment with single dose ivermectin. The transparencies were coded and the lesions scored by an uninvolved investigator. There was a marked and highly significant ($p < 0.01$) improvement in dermatitis three months after treatment when compared to that immediately before therapy.

These data show that serious adverse reactions are rare with ivermectin during community-based treatment. Major adverse reactions were not seen, even in high risk subjects, and mild reactions were seen even less commonly on re-treatment. Re-challenge of persons who experienced a reaction with first treatment did not reproduce the reaction, arguing against an idiosyncratic or allergic reaction. Ivermectin therapy has a beneficial effect on onchodermatitis three months after treatment; more long term effects are under study. Ivermectin thus shows promise as the first means of chemotherapy-based control of onchocerciasis on a mass scale.

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EFFECT OF MASS TREATMENT OF A HUMAN POPULATION WITH IVERMECTIN ON TRANSMISSION OF ONCHOCERCIASIS IN LIBERIA, WEST AFRICA

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The impact of mass treatment with ivermectin on the intensity of *Onchocerca volvulus* transmission by the black fly, *Simulium yahense*, was evaluated on the Liberian Agricultural Company rubber plantation in Liberia, West Africa. The drug was administered at two annual intervals reaching 58 - 60 % of the approximately 14,000 people living in 73 camps. Reduction in the number of infected flies from treated areas with developing larvae (L₁, L₂, L₃ stages) of *O. volvulus* was by 93.4 % - 95.0 % and in infective flies (with L₃ larvae) was by 81.7 % - 89.3 %. Monthly transmission potential (MTP) showed a similar decrease from 22.9 to 5.8 (74.6 % reduction) in the treated area, and from 210.0 to 158.8 (24.4 % reduction) in untreated areas. These studies suggest that mass treatment of persons with ivermectin efficiently controls, and may at least temporarily interrupt transmission of *Onchocerca volvulus* by black fly vectors.

R: FILARIASIS - ONCHOCERCIASIS AND IVERMECTIN

402 TRANSMISSION BLOCKING ACTIVITY OF IVERMECTIN IN BRUGIAN FILARIAL INFECTIONS.

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Medical Entomology Laboratory, University of Florida, Vero Beach.

Brugia malayi or B. pahangi infected, microfilaremic jirds (Meriones unguiculatus) were treated with ivermectin (Iv) at a single s.c. dose of 200 ug/kg b.w. After different time intervals, Aedes aegypti mosquitoes were fed on these treated jirds. L₂ and L₃ stages failed to develop in mosquitoes that fed on jirds up to 30 days post-treatment. After one month, numbers of L₃'s recovered from mosquitoes fed on treated B. pahangi jirds, were comparable to controls. However, numbers of L₃'s recovered from mosquitoes fed on B. malayi jirds remained significantly lower than controls two and three months after treatment, suggesting that Iv may be more effective in blocking transmission of B. malayi than B. pahangi. Iv treatment had no effect on the mean number of circulating microfilariae (mf), and mosquitoes ingested comparable numbers of mf compared to controls. Only in the case of B. malayi jirds did the circulating mf counts fall 30 days after treatment. Therefore, the failure of mf to develop to L₃ in mosquitoes fed on jirds within 30 days of treatment, was not due to failure of mosquitoes to ingest mf but could be due to the direct effect of Iv on mf. B. malayi mf also failed to develop to L₃ in mosquitoes that were allowed to feed on microfilaremic jird blood treated with Iv (50 ng/ml) in vitro, indicating its efficacy at low concentrations. In addition to N-acetyl glucosamine, mf obtained from Iv treated jirds showed D-mannose, N-acetyl galactosamine and L-fucose moieties on the surface of the sheath. Thus, surface alterations by Iv might render mf from treated jirds unsuitable for further development.

403 DOUBLE BLIND COMPARATIVE STUDY OF IVERMECTIN AND DIETHYLCARBAMAZINE (DEC) IN BANCROFTIAN FILARIASIS: ACTIVITY AGAINST THE ADULT STAGE OF THE PARASITE.

Frank Richards,* David McNeeley, Ralph Bryan, Mark Eberhard, Maryse McNeeley, Patrick Lammie, Yves Bernard, and Harrison Spencer. Division of Parasitic Diseases, CDC, Atlanta, Georgia, and Hopital Ste. Croix, Leogane, Haiti.

Post-DEC nodular lymphatic reactions are suggested to result from death of adult Wuchereria bancrofti parasites. To determine the efficacy of ivermectin against adult stages, we registered such reactions among 30 adult Haitians treated with ivermectin or DEC. To avoid effects stemming from clearance of microfilariae, all patients received 20 (u)g/kg ivermectin (Phase 1). Five days afterward (Phase 2), the 30 patients were randomly divided into 3 groups for blinded treatment: Group A - one ivermectin dose of 200 (u)g/kg; Group B - two ivermectin doses of 200 (u)g/kg separated by 24 hrs (total dose of 400 (u)g/kg); Group C - standard course of DEC (72 mg/kg over 12 days). Patients were examined daily during treatment; codes were broken 30 days after the beginning of Phase 2. There was a 99% reduction in mean nocturnal microfilarial density among the 30 patients by the end of Phase 1. Twenty-seven patients (90%) developed a mild to moderate flu-like syndrome 24 to 72 hrs after treatment. In Phase 2, 3 patients in Group C developed painful spermatic cord nodules, and another patient, inguinal canal pain. No reactions of this type were noted among patients in Groups A or B (P<.05, Fishers' Exact Test). Our findings suggest that ivermectin, unlike DEC, has little or no efficacy against the adult stage of W. bancrofti. (Supported in part by WHO Technical Service Agreement 05-181-41, NIH grant AI 16315, and Merck, Sharpe and Dohme).

S: MALARIA - PRE-ERYTHROCYTIC STAGES

404 CIRCUMSPOROZOITE PROTEIN HETEROGENEITY IN THE HUMAN
MALARIA PARASITE PLASMODIUM VIVAX.

R. Rosenberg*, R. Wirtz, D. Lanar, J. Sattabongkot, T. Hall, A. Waters, and C. Prasittisuk. AFRIMS, Bangkok, Thailand; WRAIR, Washington, D.C.; NIH, Bethesda, MD; and Ministry of Public Health, Bangkok.

The first example of phenotypic heterogeneity in the repetitive domain of a human malaria circumsporozoite (CS) protein - a major target of candidate vaccines - has been found. Over 14% of clinical vivax cases from western Thailand on whom laboratory reared Anopheles dirus were successfully infected produced sporozoites not recognized by monoclonal or polyclonal antibodies to sporozoites of four human and several simian malarias. Morphologically, the blood stages of these non-reactive (NR) parasites were identical to vivax; when tested with ribosomal DNA probes specific for the four human species, only vivax was positive. The CS genes of a NR and reactive isolate from the same site were sequenced after polymerase chain reaction amplification. The repetitive domain of the reactive was identical to previously published examples, but the NR was characterized by a nonamer unit homologous at only 3 amino acid positions. The post-repeat regions of both Thai isolates, which may contain T-cell epitopes, differed from Latin sporozoites. Monkey antibodies to a candidate vivax vaccine did not bind to NR sporozoites and it seems unlikely that a vaccine based on the repeat will be universally protective.

SEQUENCE ANALYSIS OF NATURALLY OCCURRING VARIANT CS GENE
405 REPEATS OF PLASMODIUM VIVAX.

*D.E. Lanar, R. Wirtz, and R. Rosenberg. Walter Reed Army Institute of Research, Washington D.C. and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Batches of sporozoites from mosquitos fed on patients in Thailand with Plasmodium vivax malaria were reacted in an ELISA test with monoclonal antibodies (Mab) specific for the circumsporozoite (CS) repeat of P. vivax GDRA(D/A)GQPA. About 85% of sporozoite batches reacted positively (R) at high titers; the remaining 15% did not react (NR) or gave a low reaction (LR) with the antibody. Using PCR the gene segment between region I and region II of the CS gene was cloned and sequenced from R, NR and LR batches of sporozoites. For all isolates the DNA sequence between the end of the repeat region and region II were similar to previously published P. vivax genes. However, while the R batch of sporozoites contained the gene sequence encoding the previously reported GDRA(D/A)GQPA repeat, the NR sporozoites contained only a sequence encoding a new repeat, ANGAGNQPG. The LR sporozoites contained primarily the repeat expressed in the NR sporozoites, ANGAGNQPG, but also included sequence encoding two copies of a new and different nonapeptide, ANGADDQPG. It is possible that this nonapeptide in the LR isolate has sufficient similarity to repeats of the reactive CS peptide, GDRADGQPA, to bind the specific Mab made against the CS gene repeat units. These variations in CS gene repeats have important implications for malaria vaccine development.

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406 FURTHER CHARACTERIZATION OF TARGET ANTIGENS OF MALARIA TRANSMISSION-BLOCKING IMMUNITY

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A complex of three antigens on the surface of gametes of malaria parasites (*Plasmodium falciparum*, *P. gallinaceum*) has been identified as the target of antibodies blocking infectivity of gametocytes in the mosquitoes. Most monoclonal antibodies produced so far are directed against conformational epitopes requiring intact -S-S- bonds in these antigens. We have undertaken a detailed study in *P. falciparum* to characterize the nature of this complex and accessibility of -S-S- bonds and -SH groups to various chemical treatments. Limited proteolysis and immunoprecipitation techniques are being employed to characterize the epitopes. Since two of the antigens are also slycoproteins, lectins have been employed to evaluate the role of carbohydrate in the formation of epitopes.

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PREFERENTIAL INDUCTION OF ANTIBODY SPECIFIC FOR THE REPEAT OR FLANKING REGIONS OF THE CS PROTEIN OF *P. FALCIPARUM*.

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We have studied the ability of distinct T cell epitopes of the circumsporozoite (CS) protein of *P. falciparum* to provide help for antibody formation specific for the repeat region or the flanking region of the CS protein. Mice were primed with peptides containing a T cell epitope, followed by infection with vaccinia virus engineered to express the CS protein. Antibody responses were measured by ELISA. CBA (H-2^k) mice immunized with peptide 328-343 from the Th2R region developed high level responses to both the repeat and flanking regions of the CS protein. BALB/c (H-2^d) mice immunized with the same peptide developed high level responses to the repeat region but not to the flanking regions. Finally, C57BL/6 mice immunized with R32tet32, containing the T cell epitope from the repeat region, also developed high levels of antibody specific for the repeat region but not to the flanking regions. These findings suggest that these T cell epitopes may preferentially direct antibody responses to a particular B cell epitope. (Supported by UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases; Gustav and Louise Pfeiffer Research Foundation; Lutheran Brotherhood MD/PhD Scholarship)

S: MALARIA - PRE-ERYTHROCYTIC STAGES

- 408 CD4+ T CELL CLONES FROM A P. falciparum SPOROZOITE IMMUNIZED VOLUNTEER RECOGNIZE A T CELL EPITOPE WITHIN THE REPEAT REGION OF THE CS PROTEIN. E.H. Nardin*, D. Herrington, M. Levine, D. Stuber, P. Barr, R. Altszuler, P. Clavijo and R.S. Nussenzweig. Department of Medical and Molecular Parasitology, New York University, NY, Center for Vaccine Development, University of Maryland, Baltimore, MD., Hoffman-La Roche, Basel, Switzerland and Chiron Corporation, Emeryville, CA.

The first T cell clones to be isolated from a sporozoite immunized volunteer were found to specifically recognize the native circumsporozoite (CS) antigen expressed on P. falciparum sporozoites, as well as recombinant CS proteins expressed in E. coli and yeast. Proliferation and gamma interferon production by the CD4+CD8- T cell clones was species specific since the clones did not recognize sporozoites or recombinant CS proteins of other human, simian or rodent malarias. Synthetic peptides and recombinant proteins containing various CS domains were used to map the T cell epitope within the 5' repeat region of the CS protein. This unique epitope is contiguous, but does not cross-react with the immunodominant B cell epitope (NANP)₃ of the P. falciparum CS protein.

This work was supported by the Agency for International Development (DPE-0453-A-00-5012-00) and the National Institutes of Health (R29 AI25085).

- 409 A PROCESSING DEPENDENT EPITOPE IS EXPRESSED IN THE CS PROTEIN OF SEVERAL PLASMODIAL SPECIES. M. Tsuji*, R. S. Nussenzweig and F. Zavala. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

We characterized the specificity of a series of anti-sporozoite mAbs selected for their lack of reactivity with the repeat domain of the CS protein. It was found that these mAbs recognize Pb44, the mature membrane form of the CS protein, but they do not react with Pb54, the precursor of Pb44. Furthermore, they do not recognize any of the synthetic peptides representing the linear sequence of the P. berghei CS protein. These observations indicate that the epitope recognized by these antibodies is expressed only after the processing of the CS protein has occurred. The stability of this epitope after the treatment with several denaturing reagents suggests that this is not a conformational epitope but perhaps is generated by glycosylation or lipidation of the CS protein during processing. Most interestingly, this "processing dependent epitope" is present not only in P. berghei, but also in P. falciparum, P. yoelii and P. brasilianum.

This work was supported by the Agency for International Development (DPE-0453-A-00-5012-00) and the National Institutes of Health (R29 AI27458).

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EFFECT OF INGESTED ANTI-SPOROZOITE ANTIBODIES ON
SUBSEQUENT SPOROZOITE TRANSMISSION BY MOSQUITOES.

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University of Maryland School of Medicine, Baltimore, MD¹, Walter Reed Army Institute
of Research, Washington D.C.², Biomedical Research Institute, Rockville, MD³.

When ingested by malaria-infected *Anopheles stephensi* mosquitoes, anti-sporozoite antibodies (IgG) cross the mosquito midgut and bind to malaria oocysts and sporozoites developing within the mosquito hemocoel. To examine more closely the consequences of this interaction, we maintained *Plasmodium berghei* ANKA-infected *A. stephensi* mosquitoes on anti-sporozoite antibodies (i.e., immune mice) throughout parasite sporogony. Control mosquitoes were also infected but were maintained on naive (i.e., nonimmune) mice. Feeding infected mosquitoes on anti-sporozoite antibodies had negligible effect on the course of infection in the mosquito, no demonstrable reduction in the numbers of sporozoites in the salivary glands, and no reduction in the ability of immune-fed mosquitoes to transmit sporozoites to naive mice. However, sporozoites transmitted by the bites of immune-fed mosquitoes infected sporozoite-immune mice (i.e., appearance of blood stages) whereas sporozoites transmitted by control mosquitoes did not. Thus, ingestion of anti-sporozoite antibodies by infected mosquitoes seemed somehow to produce more virulent mosquito-borne sporozoites capable of infecting otherwise immune animals.

Technical assistance provided by D.O. Hayes³, M. Murphy³, V. Harrod³ and H. Wynn². Financial support provided by UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, and AID Grant DPE 6453-C-3051.

- 411 HUMAN HEPATOCYTE MEMBRANE PROTEINS 20 KD AND 55 KD SPECIFICALLY BIND
TO PLASMODIUM FALCIPARUM SPOROZOITES. J. van Pelt, J. Kleuskens, M.
Schepens, J-P. Verhave, S.H. Yap, M.R. Hollingdale. University of
Nijmegen Medical School, The Netherlands, and The Biomedical
Research Institute, Rockville, MD.

To determine which proteins on human hepatocytes may be involved in the invasion of *Plasmodium falciparum* sporozoites, human liver membrane proteins were extracted with CHAPS and labelled with ¹²⁵I/IODOGEN. After incubation of radiolabelled membrane proteins with *P. falciparum* sporozoites, two proteins, 20 KD and 55 KD, were detected by SDS/PAGE autoradiography, purified by electrophoresis, and labelled with ¹²⁵I/IODOGEN. Binding of these proteins with *P. falciparum* sporozoites was greatly reduced by an excess of the corresponding non-labelled proteins, but not by membrane proteins from non-hepatic cells. *P. falciparum* sporozoite invasion into human hepatocytes was partially inhibited in the presence of these purified proteins, but *P. falciparum* sporozoite invasion of human hepatoma (HepG2-A16) cells was completely inhibited even at low concentrations (<5µg/ml). Sheep antisera raised against the 55 KD protein reacted on western blots only with the human hepatocyte 55 KD protein, and gave no cross-reaction with rat liver membrane or human kidney membrane proteins (cells not invaded by *P. falciparum* sporozoites). Anti-55 KD antiserum did not cross-react with the 20 KD protein. These results support our earlier results that sporozoite invasion of hepatic cells is mediated by specific receptors.

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- 412 RIBOSOMAL SWITCH AFTER INVASION OF PLASMODIUM BERGHEI SPOROZOITES IN CULTURED HEPATIC CELLS. J. Zhu, A. Appiah, T. F. McCutchan, A.P. Waters, A. Lal, M.R. Hollingdale. Biomedical Research Institute, Rockville, MD; laboratory of Parasitic Diseases, NIAID, Bethesda, MD.

Two stage-specific malarial ribosomal genes (A-gene specific for blood stage, C-gene specific for sporozoites) have been described previously. However, the precise time point of the switch from C- to A-specific ribosomal RNA has not been determined experimentally during the malarial cycle, and it is unknown whether such a switch represents the stability of two different rRNA's in different developmental stages or differential production of the two transcripts. We previously reported the use of stage-specific rRNA probes to quantitate sporozoite invasion and EE development of P. berghei in human hepatoma (HepG2-Al6) cells. We report here more detailed studies on the switch of ribosomal RNA's after P. berghei sporozoite invasion of HepG2-Al6 cells. The switch from C-gene to A-gene expression may occur within three hours after invasion, and by 16 hours the mature form of A-specific rRNA is predominantly present as demonstrated by Northern hybridization. The dominance of A-rRNA is due to the increased production of the transcripts. The switch from C-gene to A-gene was not prevented by irradiating sporozoites at 8-kRads. Since irradiated sporozoites elicit complete protection in mice to sporozoite challenge, this suggests that the EE-specific translation apparatus remains intact, and expression of EE-specific antigens may play an important role in protection elicited by irradiated sporozoites.

- 413 PRODUCTION, PURIFICATION AND CHARACTERIZATION OF A HUMAN MONOCLONAL IgM ANTIBODY TO THE REPEAT REGION OF PLASMODIUM FALCIPARUM CS PROTEIN. S. Futrovsky, M.R. Hollingdale, J. Sadoff, L. Iccayan, A. Appiah, D. Monheit, W.R. Ballou, J. Chulay, D.M. Gordon. WRAIR, Washington, DC; Biomedical Research Institute, Rockville, MD; Univax Corporation, Rockville, MD.

Passive transfer of MAb's directed against the CS repeat B-cell epitope of rodent and primate malarias have conferred complete protection against sporozoite challenge. PBL's were obtained from a human volunteer immunized with the recombinant R32tet₃₂ CS vaccine by Ficol-hypaque separation, transformed with EBV and fused with the SHMD33-0 heteromyeloma cell line. Cultures were screened for production of antibody to R32LA by ELISA. Culture supernatants were concentrated by ultrafiltration, and further purified by ion exchange chromatography or gel filtration. Fractions were tested for ELISA or western blot reactivity with NANP antigen and the presence of IgM antibody in reactive fractions confirmed. Culture supernatants reacted with P. falciparum sporozoites by IFA, and at a dilution of 1/10 had ISI reactivity of 85%. After ultrafiltration, concentrated antibody strongly reacted by IFA, and when diluted to 1/10, had ISI activity of 94 to 98%. Purified IgM antibody from ion exchange completely blocked sporozoite invasion, although gel filtration proved less effective in purifying IgM antibodies. Current research is to develop and characterize human IgG MAb's as potential anti-malarial immunoprophylactic agents.

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- 414 IMMUNOLOGICAL CHARACTERIZATION OF A RECOMBINANT PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN DEVOID OF REPEATS. M. Goss, D.M. Gordon, C. Silverman, G.F. Wasserman, M.C. Seguin, D. Sylvester, M.R. Hollingdale. Smith-Kline & French Laboratories, Swedeland, PA; WRAIR, Washington, DC; BRI, Rockville, MD.

The circumsporozoite (CS) protein of Plasmodium falciparum has been the primary target for a vaccine against the sporozoite stage of this parasite. Human clinical trials to date have utilized the immunodominant B-cell epitope, the repeating NANP (NVDP) tetrapeptide as a vaccine candidate. Aside from possibly providing T-helper epitopes, little is known about the role of CS non-repeating flanking regions in inducing protective immunity. We therefore constructed a recombinant molecule which contains both the N-terminal and C-terminal portions of the CS protein expressed in conjunction with 81 amino acids from the nonstructural protein (NS1 of influenza A. This recombinant protein, NS1₈₁RLFd9, represents the P. falciparum CS protein minus the repeating tetrapeptide region along with the nine amino acids just distal to the repeats. In contrast to immunogenicity studies with the repeating CS region, in which only H-2^b mice responded, immunogenicity studies using NS1₈₁RLFd9 demonstrated varying degrees of positive responsiveness in A/J (H-2^d), C57B1/6 (H-2^b), and Balb/c (H-2^d) mice. Rabbits produced high titer antibodies to this molecule which inhibited sporozoite invasion (ISI) of hepatoma cells in vitro. Using these sera we have mapped potential regions of the flanking sequences which elicit ISI antibodies.

- 415 PLASMODIUM FALCIPARUM SPOROZOITE ANTIGEN ASSOCIATED WITH PROTECTION OF MICE TO P. BERGHEI SPOROZOITE INFECTION. B. Sina, W. Weiss, V. Harrod, V.E. do Rosario, S. Aley, D. Hayes, J.F.G.M. Meis, M.R. Hollingdale. The Biomedical Research Institute, Rockville, MD; NMRI, Bethesda, MD; University of Nijmegen Medical School, The Netherlands.

Mice were immunized bi-weekly by bite of P. falciparum-infected mosquitoes and between 40-90% were protected against challenge with P. berghei sporozoites. Protection was specific, as P. falciparum-immunized mice were not protected against challenge with P. yoelii sporozoites. Level of protection depended upon the numbers of exposures to P. falciparum sporozoites. The contribution of specific antibody, CD4⁺ and CD8⁺ cells to protection against challenge was examined in passive transfer and depletion experiments. A 42 KD P. berghei sporozoite non-CS protein was detected by western blots with pre-challenge sera from mice protected to P. berghei sporozoite challenge. Sera from rabbits immunized similarly by bite of P. falciparum or P. berghei-infected mosquitoes were reacted heterologously in western blots with P. falciparum and P. berghei sporozoites, and detected an apparently similar 42 KD protein in both species. Both rabbit and mouse anti-P. falciparum sporozoite sera blocked P. berghei sporozoite invasion of hepatoma (HepG2-A16) cells and reacted with sporozoites and EE parasites by immunoelectron microscopy. This non-CS antigen common to both P. falciparum and P. berghei, thought to be a protective antigen, is being further characterized.

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- 416 MHC-unrestricted antibody formation to repetitive malaria antigens.

Louis Schofield and Patrick Uadia, Department of Medical & Molecular Parasitology, New York University School of Medicine, New York, New York.

Antibody formation to the repetitive domain of the circumsporozoite protein of malaria sporozoites has been thought to require MHC-restricted cognate interaction among antigen-specific T and B cells. In this study, we demonstrate that, in contrast to synthetic peptide and recombinant CS vaccines, an MHC-unrestricted, factor driven (non-cognate) mechanism of T/B cooperation occurs in response to whole sporozoites, driving antibody production to the repeats. These observations allow us to propose a selective (Darwinian) rationale for the repetitive domains found within protein antigens of eukaryotic parasites such as malaria.

CLINICAL TROPICAL MEDICINE GROUP MEETING (No abstracts available)

- 417 NEW TRENDS IN THE DIAGNOSIS AND TREATMENT OF FILARIASIS. E.A. Ottesen. Laboratory for Parasitic Diseases, National Institutes of Health, Bethesda, MD.
- 418 UNKNOWN CASE PRESENTATIONS IN CLINICAL TROPICAL MEDICINE. F.J. Bia, J.S. Keystone, and M. Wittner. Yale University School of Medicine, New Haven, CT; Toronto General Hospital, Toronto, CANADA; and Albert Einstein College of Medicine, New York, NY.
- 419 MALARIA UPDATE 1989: MEFLUQUINE SAFETY AND EFFICACY. C.C. Campbell and H.O. Lobel. Malaria Branch, Centers for Disease Control, Atlanta, GA.
- 420 BUSINESS MEETING. E. Jong. University of Washington, Seattle, WA.

30TH ANNUAL OPEN MEETING OF THE AMERICAN COMMITTEE
ON ARTHROPOD - BORNE VIRUSES
(No abstract available)

421 Nat Young Award

SCIENTIFIC SESSION: APPLICATION OF MOLECULAR TECHNIQUES TO
ARBOVIRUS EPIDEMIOLOGY

422 INTRODUCTION. R.E. Shope. Yale Arbovirus Research Unit, Yale
University, New Haven, CT.

423 RNA VIRUS EVOLUTION AND EPIDEMIOLOGY DETERMINED BY LIMITED GENOMIC
SEQUENCING. R. Rico-Hesse. Yale Arbovirus Research Unit, Yale
University, New Haven, CT. (No abstract available)

SCIENTIFIC SESSION: APPLICATION OF MOLECULAR TECHNIQUES TO
ARBOVIRUS EPIDEMIOLOGY

- 424 ENVIRONMENTAL RELEASE OF ORGANISMS WITH MOLECULAR MARKERS. D.H.L. Bishop, NERC Institute of Virology, Oxford, UK. (No abstract available)

- 425 NUCLEOTIDE SEQUENCES OF THE 26S RNAs OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUSES P676, 3880, and EVERGLADES.

*R.M. Kinney, J.M. Sneider, K.R. Tsuchiya, and D.W. Trent. Centers for Disease Control, Division of Vector-Borne Viral Diseases, Fort Collins, CO.

We have previously reported a comparative sequence analysis of the complete 42S RNA genomes of VEE Trinidad donkey (TRD) virus and its attenuated vaccine derivative, TC-83 virus. Nine of the 11 nucleotide mutations occurred in the 26S RNA region (encoding the structural proteins) of the genome. We have recently sequenced the 26S RNA regions of epizootic P676 virus and two structurally and antigenically related enzootic VEE viruses, 3880 and Everglades (EVE). Relative to TRD virus, we identified 138, 220 and 357 nucleotide differences which translated to 20, 22, and 53 amino acid changes in the 26S RNAs of P676, 3880, and EVE viruses, respectively. Although 12, 15 and 30 amino acid differences occurred in the E2 glycoproteins of P676, 3880 and EVE viruses, only 7, 8 and 17 differences occurred in the E2 ectodomain regions, respectively. P676, 3880 and EVE viruses shared several common amino acid substitutions in E2. The E1 glycoproteins of P676, 3880 and EVE viruses were more highly conserved, showing only 1, 2 and 7 amino acid differences, respectively, relative to TRD virus E1. The high degree of sequence conservation in the structural proteins of TRD, P676, 3880 and EVE viruses supports the hypothesis that epizootic VEE virus may originate by mutation in enzootic VEE virus populations.

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427 EPIDEMIC REPORTS.

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- 428 EVALUATION OF CLINICAL CASE DEFINITIONS FOR MALARIA IN URBAN, CENTRAL AFRICA. *N. Shaffer, K. Hedberg, F. Davachi, L. Bongo, A. Vernon, B. Miaka, P. Nguyen-Dinh, J.G. Breman. Malaria Branch, Centers for Disease Control, Atlanta, GA, USA; Mama Yemo Hospital and PEV/CCCD, Kinshasa, Zaire.

Fever is the main criterion used to diagnose and treat malaria in Africa. To test the validity of this approach in an urban area with endemic malaria, we evaluated different clinical case definitions for malaria. 1165 (95%) of 1225 children <14 years of age presenting to a large hospital emergency ward in Kinshasa, Zaire during 2 weeks in November 1988 were enrolled. By history, 75% of children enrolled had fever, 60% had cough and 51% had received antimalarial treatment. On examination, 59% had an elevated respiratory rate ($>50/\text{min}$), 50% had an axillary temperature $>37.5^\circ\text{C}$, and 16% had a palpable spleen. 21% were parasitemic with Plasmodium falciparum (GMPD of positives = $9321/\text{mm}^3$). As a predictor of parasitemia, history of fever was sensitive (87%), but not specific (27%), and had a low positive predictive value (24%). If history of fever were to be used as the sole criterion for giving antimalarial therapy, 76% of children in this setting would be treated, with a diagnostic accuracy of 40% and a treatment accuracy of 24%. Specificity increased but sensitivity decreased when factors associated with parasitemia in this population (anemia or palpable spleen) were included in the clinical case definition, or when the presence of symptoms suggestive of other diseases (cough and rapid breathing) were used to exclude possible cases. Fever remains a useful clinical definition for malaria when used to screen for a potentially severe disease. However, fever alone is a poor predictor of parasitemia, and may contribute to overuse of antimalarial drugs and undertreatment of other illnesses. (Supported by USAID PASA BAF 0421 PHC 22333).

- 429 EPIDEMIOLOGICAL MODELS FOR SCHISTOSOMIASIS: A VARIABLE RATE APPROACH
A. D. Long, Agency for International Development, Washington, D.C.

Epidemiological models for schistosomiasis are critically reviewed with special attention given to Muench's catalytic models. Prevalence data from Riche Fond Valley, St. Lucia, from 1968 to 1978, are presented and used to test the two-stage constant rate model. This cohort model approach is frequently used in conjunction with cross-sectional data under the assumption of an endemic steady state. However, when tested against sequential sets of either cohort or cross-sectional data, parameter estimates were found to vary, indicating that the assumption of an endemic steady state was not valid, making use of catalytic constant rate models questionable for schistosomiasis.

A deterministic mathematical model for the epidemiology of schistosomiasis is then presented which projects prevalence as a function of age and time. The model is an application of the McKendrick-von Foerster equation to infectious disease epidemiology. It is a further development of Muench's two-stage catalytic model, but differs from Muench's model in that it incorporates variable conversion and reversion rates. Riche Fond Valley data are used to calibrate and test the model. The model is a set of simultaneous, partial differential equations--one for the proportion uninfected and one for the proportion infected--which are treated as difference equations and solved iteratively. Linear regression equations for age- and time-specific conversion and reversion rates are incorporated into the model. Analysis of the residuals indicates that the model provides an excellent method of projecting prevalence. Color enhanced Modified Lexis Diagrams of the prevalence, conversion and reversion data are presented.

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- 430 INJECTION AS A RISK FACTOR FOR PARALYTIC POLIOMYELITIS
R.W. Sutter*, P.A. Patriarca, S.L. Cochi, and S. Brogan. Centers for Disease Control, Atlanta, GA, and Ministry of Health, Muscat, Oman.

It has been postulated that injections administered to persons incubating poliomyelitis may provoke paralytic disease. Between January 1988 and March 1989, 119 cases of paralytic poliomyelitis were reported in Oman, for a crude attack rate of 9/100,000 total population. Nearly 80% of case-patients were younger than 2 years of age; the age-specific attack rate in children 6-11 months of age was 148/100,000. The outbreak provided a unique opportunity to study the role of DTP injections as a risk factor for paralytic disease using matched case-control study methods. Sixty cases 5-24 months of age were enrolled, each matched with 10 controls for age and village of residence. Vaccination histories were obtained from health center registries. Information on other intramuscular injections was not collected. A significantly higher proportion of cases (47% [28/60]) received a DTP injection within 30 days prior to onset of paralysis, odds ratio=2.0 (95% confidence interval=1.4-3.6), than their age-matched controls (30% [18/59]). Based on this finding, the proportion of cases that may have been provoked by DTP injections (i.e. population attributable risk) was 23%. In addition, the anatomic site of injection corresponded to the anatomic site of paralysis in 90% of 20 case-patients for whom the site of injection was known. Our findings provide support for the hypothesis that DTP injections have the potential to provoke paralysis in persons already incubating poliomyelitis. Whether this may be related to DTP vaccine or the injection itself could not be determined.

- 431 *THE INFLUENCE OF HOUSEHOLD CHARACTERISTICS ON DIARRHEA OCCURRENCE IN RURAL EGYPT*

*Mostapha Habib, Charles E. Wright, Mohamed El Alamy, Herbert L. DuPont
Epidemiology Study Center-Bilbeis, Egypt and The University of Texas Health Science Center, Houston, TX. Supported Diarrheal Disease Project, 03-340-C, Centers for Disease Control, Atlanta, GA

In rural Egypt 317 households, during a 12-month period of time during 1981-1982, were prospectively studied for the occurrence of diarrhea (by twice weekly home visits) and for selected environmental factors. The range of household incidence rates of diarrhea ranged from 19.5 per infant year to 0 episodes with an average of 4.8 episodes per infant year. Through applications of stepwise multiple regression, a number of household variables were significantly ($P<0.05$) associated with increased rates of diarrhea in household infants: house with poor state of repair, incomplete ceilings, dirt floor in main living area, single living area for eating, child rearing, etc; stored food present on survey; use of well water versus tap water for cooking and bathing; not covering infant with veil while sleeping; and reporting many rodents in the house. Other factors not significantly linked to diarrhea included: lack of electricity or refrigeration; presence of indoor privy or latrine; free movement of animals in the home; using animal feces for cooking fire fuel; and maintaining a household infant in an outside day care facility. It was found that 25% of the total variance in incidence of diarrheal illness was explained by the environmental variables under study. The observations suggest that environmental interventions may be useful in preventing up to one-fourth of diarrhea occurrences in infants living in rural Egypt.

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ETIOLOGY OF UPPER RESPIRATORY DISEASE AMONG CHILDREN IN CAIRO, EGYPT.

432 *R.L. Haberberger, N. Osman, R. Elyazeed, and D.M. Watts. U.S. Naval Medical Research Unit No.3, Cairo, Egypt; Al-Azhar Women's University, Cairo, Egypt; and Ministry of Health, Assiut, Egypt.

Three hundred sixty eight children were enrolled in a two year case-control, pilot study to determine the etiology of upper respiratory disease in Cairo, Egypt. The children ranged in age from 1 day to 12 years of age. Sixty percent of the children were males, forty percent were female. The study subjects were divided into three groups: upper respiratory infection (URI), URI with diarrhea, and diarrhea only. The isolation of Streptococcus pneumoniae from nasopharyngeal specimens was significantly associated with URI and URI with diarrhea. When compared to bacterial agents, none of the seven viral agents (respiratory syncytial virus, adenovirus, influenza A, B, and parainfluenza 1, 2, and 3) examined were significantly associated with disease. Only two subjects had mixed bacterial and viral infections. Upper respiratory bacterial pathogens were significantly associated with diarrhea. Identification of a viral agent was frequently associated with hoarseness of voice but only approached significance ($P=0.0735$). Fever was significantly associated with identification of a viral agent ($P=0.052$). Asthma was associated with viral identification ($P=0.06$). Cyanosis at birth was strongly associated with viral infection ($P=0.04$). The presence of abdominal tenderness was significantly associated with the isolation of bacteria ($P=0.026$). To our knowledge this is the first comprehensive study of the etiology of acute upper respiratory illness of children in Egypt. Our findings will be used to identify public health measures which may aid in the reduction of upper respiratory disease among children in the developing world. (Supported by NMRDC, Bethesda, MD, Work Unit No. 3M162770A870.AR.322)

433 CLINICAL-EPIDEMIOLOGIC STUDY OF CHAGAS' DISEASE IN BOLIVIA. M. Pless*, D. Juraneck, P. Kozarsky, F. Steurer, G. Tapia, H. Bernudez. Emory University School of Medicine, Atlanta, GA; Parasitic Diseases Branch, Center for Disease Control, Atlanta, GA; Chagas Laboratory, San Simon University, Cochabamba, Bolivia.

Although Chagas' disease is thought to be a significant health problem in many parts of Bolivia, few population-based studies of prevalence or morbidity have been conducted. In June of 1988, we carried out a clinical/epidemiologic study in a rural village near Cochabamba. 96% (153) of 160 persons greater than 5 years of age participated in the study. One hundred twelve (73%) villagers were IgG seropositive (titer $\geq 1:128$) to T. cruzi by the IIF test, and 117 (76%) were positive (titer $\geq 1:32$) for IgM antibodies against T. cruzi. All villagers 35 years of age or older were seropositive. EKG conduction abnormalities were observed in 22 (20%) seropositive and 2 (5%) of seronegative villagers ($p < 0.05$). 41% of the conduction abnormalities occurred in people < 40 years of age. Complete right bundle branch block accompanied by left anterior or left posterior hemiblock occurred in 11 of 22 seropositive villagers with a conduction defect. Two additional villagers had complete right bundle branch block as the only conduction defect. 23% of villagers had symptoms and signs of heart failure and 16% had swallowing times > 10 seconds. Live reduviids (T. infestans) were found in 34 of 40 houses in the village; eggs or exuviae were found in the remaining 6 houses. Only 50% of villagers knew that reduviids were a source of infection. Data from the study suggest that T. cruzi infection may be hyperendemic in this region and is a significant cause of morbidity. A health education program coupled with a low-cost vector control program could have a significant public health impact on the disease in this region.

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- 434 **TRANSMISSION AND EPIDEMIOLOGY OF MALARIA IN MADAGASCAR.** J. P. Lepers, D. Fontenille, *P. Deloron, M. D. Andriamangiatiana-Rason, P. Coulanges. Institut Pasteur de Madagascar, Antananarivo and INSERM Unité 13, 75019, Paris, France.

Since 1988, the malaria research unit of the Madagascar Pasteur Institute investigates malariological parameters (entomological, parasitological, and immunological) in 2 areas of the island, where different patterns of malaria transmission occur. The first area is a village of the Highland Plateaux, located near Antananarivo (the capital city of Madagascar), where *falciparum* malaria was reintroduced recently and appears to be epidemic since 1986. The second study site is located on the East coast, in the Sainte Marie touristic area, which is considered as hyperendemic for malaria. In both areas, more than 90% of the malaria cases are due to *P. falciparum*. The parasitological and immunological parameters were investigated during a cohort study. 5 to 15 years old schoolchildren were followed monthly. Entomological surveys were conducted monthly for at least one year in each site.

In the Sainte Marie area, malaria is transmitted perennially, the vectors being *A. gambiae* s.s. and *A. funestus*. Sporozoite indexes vary from 0.5% to 5%, according to the village and the season. The number of infective bites per man and per year is ≈ 20 . Parasite and splenomegaly rates are relatively constant at 60 to 70%, with a gametocytic index of 10%. Almost 100% of the children have total antibody directed against *P. falciparum*, as detected by IFA. In the Highland Plateaux area, malaria is transmitted discontinuously, the vectors being *A. arabiensis*, *A. funestus*, and probably *A. gambiae* s.s.. The number of infective bites per man and per year is ≈ 1 to 2. Parasite rates vary from 40% to 60%, peaking in May. Splenomegaly rates are constantly at 35-55%, and gametocytic index is at 10%. By IFA, approximately 75% of the children have anti-*P. falciparum* antibody.

- 435 **HYPERENDEMIC HUMAN AND BOVINE FASCIOLIASIS IN A RURAL BOLIVIAN COMMUNITY.** R.T. Bryan*, J. Bjorland, C. Espindola, M. Lagrava, W. Agreda, D. Everaert, M. Vilca, A. Quiton, M. Soler, and G. Hillyer. Parasitic Diseases Branch, CDC, Atlanta, GA; Proyecto Danchurchaid, PIL, Cordepaz, La Paz, Bolivia; Ministry of Health, Bolivia; Foster Parents Plan International, La Paz; University of Puerto Rico, San Juan.

Published accounts of community-based studies of fascioliasis in Latin America are rare. Increasing recognition of clinical cases by local health care providers led to an epidemiologic investigation of fascioliasis in a rural area of the northwestern Bolivian altiplano. An unusually high prevalence of infection with *Fasciola hepatica* was identified among humans and livestock in the Aymara Indian community of Coropata. Cross-sectional surveys of the human and livestock populations were conducted in August and October 1988, respectively. Serologic specimens, fecal specimens, and questionnaire data were obtained from 46 randomly selected households. Combining serologic and coproparasitologic results, the overall prevalence of infection with *F. hepatica* was 40% in humans and 60% in cattle. Human cases clustered geographically and prevalence was highest in persons less than 20 years of age and in those who consumed certain local aquatic plants. Additional intestinal parasites identified in human stool specimens included: *Entamoeba histolytica* (31%), *Trichuris trichiuria* (26%), *Ascaris lumbricoides* (9%), *Giardia lamblia* (9%), and *Taenia* species (6%). Ecologic conditions and animal husbandry practices in Coropata are ideal for continued transmission of *F. hepatica*; current control measures may be inadequate. Further studies of morbidity, economic burden, and public health impact are needed in order to design and implement more effective control strategies.

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- 436 An Epidemiological Study of the Prevalence and Symptoms of enterobius vermicularis Infections in a Lima, Peru Shanty Town

Robert H. Gilman, Grace S. Marquis and Elba Miranda
The Johns Hopkins University, Universidad Peruana Cayetano Heredia, and
Instituto de Investigacin Nutricional

We determined the prevalence rates of pinworm infection and its relation to symptoms in 896 individuals, from 200 families, living in a Lima, Peru, pueblo joven. The Graham scotch tape test was used to detect infected individuals. Only subjects with 2 consecutive tests were included in the analysis. Seventy one children, positive for pinworm, were treated and followed for 6 months to determine their rate of reinfection.

Rates of pinworm infection were lowest in children from 0-2 years of age and highest (42%) in children age 6-10 years. Perirectal itching was the most common symptom reported in infected individuals (15%) however it was equally frequent (11%) in non-infected subjects. Other symptoms, such as bed wetting and teeth grinding were equally distributed in infected and non-infected subjects. Over 73% of children under five that had been treated were reinfected, at a linear rate, during the six month observation period.

This study demonstrates that pinworm infection is a common infection in a Peruvian pueblo joven. Yet, we could not confirm that pinworm infection was a major source of symptoms, such as peri-rectal itching or enuresis in this community. Moreover, if treatment is given to children the majority of them will be rapidly reinfected.

- 437 CHICKENPOX IN THE U.S. ARMY: A DEVELOPING EPIDEMIC OF MAJOR PROPORTIONS
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Varicella is a reportable disease in active duty personnel. A total of 4,900 Army hospital admissions for the years of 1980-88 were reviewed in order to identify trends of illness and military impact (ICD-9 codes 052.0-052.9). A 2-4 fold increase in varicella incidence was documented for the last three years at several of the installations where recruit training is conducted. An increased risk was found for recruits, lower ranks, and hispanics/blacks:

Variable	Incidence (per 100,000)	Relative Risk (95% C.L.)
Trainee	140.4	1.72 (1.43, 2.06)
Non-trainee	81.8	1
Enlisted	71.5	10.78 (8.26, 14.07)
Officers	6.6	1
Hispanic	362.3	3.68 (2.96, 4.57)
Black	163.8	1.66 (1.45, 1.91)
White	98.5	1

Disease severity was similar regardless of sex, rank, ethnicity, or age as measured by the proportion of personnel returned to normal military duties (99.6%) as well as the average number of hospital days and convalescent days per soldier (6 and 7 days respectively). Estimated direct annual hospital costs exceeded 1 million dollars. Varicella is an increasingly significant disease in the military. Strategies for prevention, including vaccination, need to be considered.

SYMPOSIUM: INTERNATIONAL TRAVEL MEDICINE

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- 439 MALARIA CHEMOPROPHYLAXIS: ADVANTAGES AND DISADVANTAGES.
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- 440 PANEL DISCUSSION: MALARIA PREVENTION.
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- 441 TRAVELERS' DIARRHEA.
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H.L. DuPont, University of Texas Medical School, Houston,
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- 443 THE EPIDEMIOLOGICAL BASIS FOR VACCINATION OF TRAVELERS.
R. Steffen, University of Zurich Medical School, Zurich,
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- 444 PANEL DISCUSSION: VACCINATION STRATEGIES FOR TRAVELERS
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R. Steffen, University of Zurich Medical School, Zurich,
SWITZERLAND.
M. Barry, Yale University School of Medicine, New Haven, CT.
E. Jong, University of Washington Medical School, Seattle,
WA.

445 **Cloning of a Highly Repeated Protein Located in the Gut
of Filarial Parasites**

L. A. McReynolds¹, C. Poole¹, G. Grandea¹, R. Maizles², M. Selkirk³ and R. Jenkins⁴.

¹New England Biolabs, Beverly, MA., ²Department of Pure and Applied Biology,

³Department of Biochemistry and Imperial College of Science and Technology, London,

UK., ⁴London School of Hygiene and Tropical Medicine, London, UK.

Using sera from dogs immunized with irradiated *D. immitis* L3s, a lambda gt11 recombinant was isolated from a cDNA library of *D. immitis*. Southern blot and DNA sequence analysis of *D. immitis* genomic DNA with the recombinant probe shows that the DNA is organized as a direct tandem repeat of twenty or more 399 bp units. Antisera prepared against the cloned *D. immitis* recombinant cross-reacts with a Bolton-Hunter iodinated protein of *Brugia malayi*. Gel analysis of the labeled protein gives a series of "ladder" like bands that are about 14-15kD apart. A very similar "ladder" like banding pattern is seen on Western blots using an n-octyl-glucoside extract of *D. immitis* worms probed with antisera to the cloned recombinant. The cloned antigen has been localized in the microvilli of the intestines of *Brugia malayi*. Immunogold localization of the antigen was determined by electron microscopy of parasite sections. The synthesis and antigenic properties of this unusual protein are under investigation.

446 **IN SITU HYBRIDIZATION: A METHOD OF RELATING CLONED ANTIGENS TO
THEIR SITE OF EXPRESSION WITHIN INDIVIDUAL FILARIAL WORMS AND
LARVAE.**

*F.B. Perler, D. Abraham, M. Mulligan, J. B. Lok, and R. Tuan. New England Biolabs, Inc., Beverly, MA, and Thomas Jefferson University and U. Pennsylvania, Philadelphia, PA.

It is often difficult to relate a cloned gene to the parasite, especially when the sequence is unlike previously identified proteins. Infective larval (L3) and L4 antigens would be ideal targets for vaccine development, but identification of cloned *Onchocerca volvulus* (OV) larval antigens has been complicated by a lack of parasite material. The technique of *in situ* hybridization of cloned DNA to mRNA in parasite sections provides a simple, fast method of determining the tissue localization of cloned antigens and whether these genes are expressed in L3 or L4. The technique is exquisitely sensitive and permits the detection of specific mRNAs in single embryos, larvae and adults. Adult *B. malayi* females were probed with 2 previously characterized cloned *B. malayi* L3 antigens (BM19 and BM22). Live worms were fixed, embedded, sectioned, and probed with biotin-labelled plasmids carrying the BM inserts. Hybridization was detected non-isotopically, using streptavidin-alkaline phosphatase and BCIP/NBT development. Sections were examined using Nomarski optics so that worm tissues and structure could be observed while positive hybridization was visualized as a dark purple stain. DNA from both clones hybridized specifically to mRNA expressed in embryos developing in the uterus, but not to other tissues of the adult worm. Single embryos were easily detected within the uterus. We are currently testing *B. malayi* L3, OV nodules, and adults, and blackflies infected with all stages of developing *O. lienalis* larvae. The combination of immunohistology and *in situ* hybridization makes it feasible to analyse the stage specific expression of numerous cloned antigens at the mRNA and protein levels using single larvae or adults.

447 **MOLECULAR GENETICS OF FILARIAL MYOSINS.**

C. Werner*¹ and T.V. Rajan.² Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx NY¹, and Department of Pathology, University of Connecticut Health Center, Farmington²

A number of investigators, working on different filarial host-parasite combinations, have shown that myosin or myosin-like proteins (including paramyosin) are recognized as antigens by a majority of individuals harbouring parasites. We have previously reported the cloning of a small genomic fragment of the tail of *Brugia malayi* myosin and its frequent immunological recognition by patients with Bancroftian filariasis, as well as the preferential recognition of a similar myosin cDNA segment from *Onchocerca volvulus* by asymptomatic individuals. This has led us to examine in detail the molecular genetics of filarial myosins, with particular reference to the number of genes and their topographical and temporal regulation of expression. We have used these probes to extend the cloned portion of the gene towards the globular head domain, the sequence of which is now substantially complete. In addition, we have isolated several *Wuchereria bancrofti* clones that hybridize to probes mapping to the S2 region of *B. malayi* myosin. The myosin genes of *O. volvulus* and *B. malayi* are more closely related to each other than either is to that of the free-living nematode *Caenorhabditis elegans*, and *O. volvulus* is closer to *C. elegans* than is *B. malayi*. The amino acid identity is roughly 70% in the rod region, and increases to over 90% towards the head. Despite this high degree of sequence conservation, the intron-exon organization of the *B. malayi* gene is markedly different from that of *C. elegans*. The *B. malayi* gene is interrupted more frequently with introns of greater length than is that of *C. elegans*. The *B. malayi* clone appears to only hybridize to one genomic fragment, whereas in *C. elegans*, the myosin family consists of four cross-hybridizing genes. The filarial myosins most closely resemble *C. elegans* MHC-B, the major body wall myosin encoded by the *unc-54* gene, rather than the pharyngeal forms.

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Expression of *Brugia malayi* 70 Kilodalton Heat Shock Genes.

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We have previously shown that *Onchocerca volvulus* 70 kilodalton heat shock protein (HSP70) is recognized by sera from amicrofilaremic individuals who live in an areas endemic for lymphatic filariasis. Using a cDNA encoding the protein to probe Southern blots and genomic libraries, we have ascertained that there are two members of the HSP70 family in *Brugia malayi*. One of these has been cloned in its entirety and pieces of this gene have been used to probe Northern blots to determine in what stages the gene is expressed. Preliminary data suggest that the gene is transcribed in microfilariae and more detailed analysis is being done to determine the kinetics of the transcription and whether it is transcribed in third stage larvae. In addition, attempts are being made to clone the second gene and use gene specific probes to determine the transcription of each of the genes independently. Understanding the timing of their expression may provide insight into the correlation we have found between recognition of the proteins by sera of amicrofilaremic versus microfilaremic patients.

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AN EXAMINATION OF THE PARAMETERS INVOLVED IN PURIFICATION OF PARASITE ANTIGEN FUSION PROTEINS.

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β -galactosidase fusion proteins have been used to investigate the properties of parasite antigens. However purification is sometimes difficult, yields of the β -galactosidase fusion protein are often low and β -galactosidase is very immunogenic. A protein purification system has been developed that utilizes affinity column purification with maltose binding protein (MBP) fusions. It is possible to purify gram amounts of fusion protein on a single step amylose affinity resin; the fusion protein can then be cleaved with factor Xa protease at a site between MBP and the antigen of interest. However not all fusion proteins are ideal; examples of proteins which tend to be insoluble or unstable in *E. coli* and general approaches to solving these problems of protein stability and degradation will be covered. Factors found to be important in fusion protein purification include choice of host bacterial strain, length of induction period and method of cell lysis. Using several filarial antigens as examples, these and other parameters will be discussed along with alternative methods of protein purification.

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ISOLATION AND CHARACTERIZATION OF REPETITIVE DNA CLONES OF *WUCHERERIA*

BANCROFTI. *N. Raghavan, S. A. Williams, E. A. Ottesen and T. B. Nutman. Laboratory

of Parasitic diseases, NIAID, Bethesda MD and Smith College, Northampton, MA.

A genomic DNA library of *W. bancrofti* was constructed in the expression vector λ gt11.

The library had a titre of 1.6×10^6 pfu/ml with less than 1% non-recombinants. The repetitive clones were isolated by hybridization of total nick translated *W. bancrofti* DNA with approximately 5×10^4 recombinants. Approximately 1% of these recombinants were repetitive, with the repeats falling into three different categories based on their hybridization profiles. 4/10 repeats showing the strongest signal with *W. bancrofti* DNA and varying copy numbers were further characterized. The clones designated λ Wb1-1, λ Wb2-1, λ Wb3-1 and λ Wb6-1 had insert sizes of 6.6 kb, 5.2 kb, 5 kb and 4.0 kb respectively. When the species specificity of the clones were determined by DNA dot blot analysis, all 4 hybridized with *Brugia malayi* and *W. bancrofti* DNA but not with *B. pahangi* or human DNA; however, at lower concentrations clone λ Wb6-1 hybridized only with *W. bancrofti* DNA. The arrangement of these repetitive sequences in the parasite genome and their potential value for developing diagnostic DNA probes for recognizing different geographical isolates or 'strains' of *W. bancrofti* are currently being investigated.

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- 451 ISOLATION AND PARTIAL CHARACTERIZATION OF REPETITIVE DNA SEQUENCES FROM Loa loa. A.D. Klion and T.B. Nutman. Laboratory of Parasitic Diseases, NIH, Bethesda, MD.

Parasitologic diagnosis of loiasis is often difficult, as many of the estimated 13 million people affected are amicrofilaremic. Similarly serologic tests are hampered by the lack of available parasite material and cross-reactivity among the various filarial species. To circumvent these problems, a genomic DNA library was constructed in λ gt11 using EcoRI digested DNA from Loa loa microfilariae isolated from 2 West African patients. The library, which has a titer of 1.42×10^6 pfu/ml, was screened with nick-translated total Loa loa DNA. Twenty highly reactive clones were selected from the 10^4 recombinants screened. None of these hybridized with nick-translated human DNA. Five of the clones (L1 1-5) were plaque purified and showed insert sizes ranging from 3.2 to 6 kb. In Southern blot analysis, 32 P-labelled L1 1 (insert size 6 kb) recognized interspersed sequences in Loa loa DNA digested with EcoRI or HhaI, but not in DNA from Brugia malayi or in human DNA. Further characterization of these and other recombinants from this library should provide the tools necessary for species-specific diagnosis of Loa loa and provide insights into the relationship between Loa loa and the other filarial species.

- 452 ISOLATION OF A MEMBER OF REPEATED SEQUENCE FAMILY SPECIFIC FOR SAVANNAH FORM O. volvulus.

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Maintenance of the control of O. volvulus would be assisted by the development of assays to identify the origin of new foci of transmission within the control region. A DNA probe, designated pFS-1, has been identified which distinguished O. volvulus from the forest region of Liberia from Mali savannah.

To complement the use of pFS-1, a second clone designated pSS-1BT has been isolated which is specific for the savannah form of O. volvulus. This clone was isolated as a subclone of a clone designated pSS-1 which hybridized preferentially but not exclusively to savannah form DNA. pSS-1 was found to consist of 2 members of a 149bp repeat sequence family present in the genome of O. volvulus, of which pFS-1 is also a member. Sequence analysis of 17 examples of the 149bp repeat unit has been used to generate a consensus sequence. A comparison of the sequence of pSS-1 to the consensus was used to identify and subclone a region of pSS-1 which appears to be completely specific for O. volvulus savannah form DNA. These results suggest that members of the 149bp repeat family have diverged during evolution, and that it is possible to isolate repeats with varying degrees of specificity. The use of these specific repeats as probes may be useful in the development of assays which may identify the geographic origin of a given O. volvulus isolate. Such information has potential utility for the Onchocerciasis Control Programme, as well as in studies investigating the existence of distinct parasite-vector complexes in different regions of Africa.

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453 PCR AMPLIFICATION AND NON-RADIOACTIVE DNA PROBES FOR THE SPECIES SPECIFIC DETECTION OF *BRUGIA* AND *WUCHERERIA* IN HUMAN BLOOD SAMPLES.

*S.A. Williams¹, J.G. Glover¹, S.J. Szabol¹, L.A. McReynolds², D. Landry², and F. Partono³. ¹Smith College, Northampton, MA; ²New England Biolabs, Beverly, MA and ³University of Indonesia, Jakarta, Indonesia.

We have applied the polymerase chain reaction (PCR) to the amplification of filarial parasite DNA in human blood samples. Specific primers have been used to amplify highly repeated DNA sequences we have characterized in *B. malayi*, *B. pahangi*, *B. timori*, and *W. bancrofti*. Microfilariae in blood samples are lysed by boiling for 15 minutes. A small aliquot of the blood sample (5 µl) is added to a 100 µl PCR reaction containing repeat specific oligonucleotide primers. Following 30 cycles of amplification, 2 µl of the PCR reaction is spotted onto nitrocellulose filters. When these samples are hybridized with non-radioactive DNA probes, samples containing as few as one or two microfilariae are easily detected. Biotinylated oligonucleotide probes can give species-specific detection of a single microfilaria in a few hours using avidin-alkaline phosphatase detection. Oligonucleotide probes labeled with the enzyme horseradish peroxidase can give the same species-specific detection with less than a 5 minute exposure to x-ray film using a chemiluminescent detection system. In a study of 45 human blood samples collected in Tanjung Pinung, Indonesia, all 45 were correctly identified by this method. This method was much faster and more sensitive than using radioactively labeled DNA probes to detect non-PCR amplified samples. The method is simple and convenient for use in endemic nations such as Indonesia and can be carried out without the use of a PCR machine.

454 FIELD TESTING OF A MONOCLONAL PROBE SPECIFIC FOR INFECTIVE LARVAE OF HUMAN *BRUGIA* PARASITES. Clotilde K.S. Carlow[~], A. Suwita[#], Z.

Bahang⁺, Purnomo[#], F. Partono[#] and Mario Philipp^{*~}. [~]New England Biolabs, Beverly, MA 01915, [#] University of Indonesia, Jakarta, Indonesia, ⁺ Ministry of Health, Jakarta, Indonesia.

Epidemiological surveys and assesment of control programmes for Brugian filariasis require the identification of natural vectors and determination of their transmission potential. This necessitates both the distinction of human Brugian parasites from the various animal filariae transmitted by the same mosquito and the evaluation of infective stages present. Neither task can be performed using morphological criteria. We have previously described the species- and stage-specific properties of a monoclonal antibody reactive with the surface of *Brugia malayi* and *B. timori* infective stage larvae (1). A simple monoclonal antibody-based immunoenzyme assay was developed (1) and we have currently investigated its applicability to field studies in an endemic area. Large numbers of wild mosquitoes consisting of 28 species belonging to 6 genera were collected in South Kalimantan, Borneo, Indonesia and examined in the field for the presence of human Brugian filariae. We evaluated the specificity and sensitivity of the assay using natural vectors and wild type *B. malayi*, *B. timori* and *B. pahangi* infective larvae. Specificity was 100% and sensitivity was consistently higher than 90%. (1) Carlow, C.K.S. et al. (1987) Proc. Natl. Acad. Sci. 84, 6914.

V: VIRUS VACCINES

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HUMORAL IMMUNITY TO VACCINIA VIRUS

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A potential drawback of vaccine strategies that involve the use of live recombinant vaccinia viruses is their obligatory linkage to the immunobiology of vaccinia virus (VAC). To address issues related to the use of such vaccines, we examined antigenic and immunogenic properties of VAC as reflected by antibody responses in mice and humans. We used monoclonal antibodies (MAbs) and murine immune sera to extend our data on serologically defined antigenic structures of VAC. To gain insight into human responses, we evaluated kinetics, magnitudes and specificities of antibody responses to VAC in 40 soldiers who volunteered to donate serum before and at three time points after their routine smallpox vaccinations. To specifically address problems of recombinant VACs, we evaluated antibody responses to VAC and to Hantaan virus (HTN) in mice immunized with recombinant VACs that expressed HTN envelope glycoproteins. Further, we tested the abilities of different VAC-specific antibodies to restrict VAC-HTN replication at the site of virus inoculation in mice, and tested whether antibody responses to HTN proteins (expressed by VAC-HTN) were reduced concurrently. Results indicated that a 26 kDa VAC surface protein is a particularly relevant target of humoral immunity, i.e., it is recognized by MAbs that neutralize VAC efficiently *in vitro* and dramatically restrict its replication in mice. Vaccinated mice, as well as humans, produced antibodies that bound VAC (in ELISA) and competitively inhibited biotinylated MAbs to the 26 kDa protein. However, it was also clear that both mice and humans produced a complex array of neutralizing antibodies, cytolytic antibodies, and antibodies with no obvious biological activities, and that antibodies equivalent to the most potent neutralizing MAbs (i.e., anti-26 kDa) were underrepresented in VAC-immune sera.

456 PROTECTION OF NONHUMAN PRIMATES AGAINST VENEZUELAN ENCEPHALITIS AFFORDED BY A RECOMBINANT VACCINIA VACCINE.

*T.P. Monath, C.B. Cropp, W. Short, R.M. Kinney, D.W. Trent, Division of Vector-Borne Diseases, Centers for Disease Control, Ft. Collins, CO, and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

The live, attenuated VEE TC-83 vaccine presently used for human and equine immunization induces a high incidence of febrile reactions and has the potential to infect mosquitoes feeding on immunized hosts. An improved vaccine was sought by insertion of the structural genes of VEE IAB (Trinidad donkey) into vaccinia virus (NY City Board of Health Strain), as described previously (Kinney, R.M. et al., J. Gen. Virol. 69:3005, 1988). The serological responses of cynomolgus monkeys immunized with the VEE-vaccinia recombinant were compared to those of monkeys given vaccinia alone, live TC-83 vaccine, or two doses of killed whole-virion TC-84 vaccine. Neutralizing antibody was detected within 10-14 days in sera from all animals receiving the three VEE vaccines, with the highest titers in those inoculated with live TC-83. Controls challenged by subcutaneous inoculation of virulent VEE developed viremia and leukopenia, whereas immunized animals did not; however, only TC-83-immunes were fully resistant, as indicated by absence of boost in antibody titer following challenge. Monkeys immunized as described above were also protected against neuroinvasion after intranasal challenge, encephalitis after intracerebral challenge.

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457 RECOMBINANT VACCINIA-VENEZUELAN EQUINE ENCEPHALITIS VIRUS (VEE) PROTECTS HORSES FROM PERIPHERAL VEE VIRUS CHALLENGE.

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Infection of equines with epizootic VEE virus frequently results in fever, leucopenia, meningoencephalitis, and death. Histopathologic changes in the hemolymphatic system include wide spread lymphoid necrosis and depletion of hematopoietic cells in the bone marrow. Changes in the CNS correlate with clinical encephalitis and include diffuse meningoencephalitis with perivascular infiltration of leukocytes, and necrosis. We have evaluated the efficacy of a recombinant vaccinia/VEE virus vaccine to protect horses against VEE. Horses immunized with the vaccinia/VEE recombinant virus developed low titre ELISA and neutralizing antibodies after the first immunization. These animals were reimmunized 92 days later with recombinant virus to boost the immune response prior to virus challenge. Serum antibody titers 7 days following reimmunization were high, indicating antigenic priming had been accomplished by the initial recombinant virus immunization. Horses immunized with the vaccinia/VEE recombinant, wild vaccinia and TC-83 virus were challenged with equine virulent VEE virus. Animals immunized with wild type vaccinia developed a profound leukopenia, became viremic, febrile and depressed, and were euthanized 6 days after challenge. Horses immunized with either the TC-83 or vaccinia/VEE recombinant viruses did not become viremic or develop clinical or hematologic signs of VEE following challenge. In both groups of immune animals, there was no significant increase in VEE antibody titer following challenge, indicating that the horses were fully protected from neuroinvasion.

458 EXPRESSION OF HANTAAVIRUS ANTIGENS FOR RECOMBINANT VACCINE DEVELOPMENT

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Hantaan virus is the prototype of the *Hantavirus* genus of Bunyaviridae and is the etiologic agent of Korean hemorrhagic fever. Hantaan and related viruses cause numerous clinically similar diseases known collectively as hemorrhagic fever with renal syndrome (HFRS). In a continuing effort to develop a vaccine for HFRS, we have examined the antigenicity and immunogenicity of Hantaan viral proteins expressed by recombinant baculo- or vaccinia viruses. Recombinant viruses were prepared that contained the complete M segment of Hantaan virus or regions of the M segment encoding only G1 or G2. Both viral expression vectors produced proteins indistinguishable from authentic Hantaan viral proteins when examined by polyacrylamide gel electrophoresis, fluorescent antibody staining, or ELISA with a variety of polyclonal and monoclonal antibodies. Mice and rabbits immunized with recombinants expressing the entire M segment developed neutralizing antibody responses. Hamsters were immunized with vaccinia-Hantaan M recombinants and later challenged with Hantaan virus. Serum antibody titers were measured by IFA and ELISA, and lung sections were examined for the presence of viral antigen. Hamsters immunized with recombinants expressing both G1 and G2 appeared to be protected from infection with Hantaan virus, but control animals immunized with vaccinia recombinants containing Hantaan S segment cDNA developed high-titered antibody responses to Hantaan virus and had antigen in their lungs. Although both expression systems show promise for future vaccine development, we have placed greater emphasis on vaccinia recombinants and have prepared new plasmid transfer vectors for use with the Connaught human vaccine strain of vaccinia virus.

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459 BLUETONGUE VIRUS SUBUNIT VACCINES

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Bluetongue virus (BTV), a member of the Reoviridae family, contains genome of 10 double-stranded RNA segments, enclosed by an inner core of five polypeptides (VP1, VP3, VP4, VP6 and VP7), and an outer capsid of two polypeptides (VP2 and VP5). The solubilised VP2 protein of virion has been shown to induce neutralizing antibodies that protect sheep against infection by BTV. In order to develop low cost subunit vaccines, cDNA representing the VP2 genes of BTV serotypes (BTV-1, -2, -10, -11, -13, -17) have been expressed in recombinant baculoviruses. High level expressions of the VP2 proteins in insect cells (*Spodoptera frugiperda*) have been obtained. The antigenic characteristics of each VP2 protein was conserved by comparison with those of the native VP2 of BTV virions. The immunogenicity and the protective capabilities of these proteins were also demonstrated. The inoculations of VP2 antigens in sheep were associated with the induction of high titers of neutralizing antibodies and sheep were produced when challenged with virulent virus. The availability of large quantities of antigenically and immunogenically reactive BTV VP2 proteins makes feasible for the safe preparation of a low cost subunit vaccine for the disease.

460 SAFETY AND EFFICACY OF A MUTAGEN-ATTENUATED RIFT VALLEY FEVER VACCINE IN PREGNANT BOVIDS. *J.C. Morrill, C.A. Mebus, R.G. Breeze, and C.J. Peters. USAMRIID, Fort Detrick, Frederick, MD and Plum Island Animal Disease Center, Greenport, NY.

A mutagen-attenuated Rift Valley fever virus vaccine (RVF MP12) has been developed which is non-pathogenic in rhesus monkeys and neonatal lambs and is non-abortogenic and non-pathogenic in pregnant ewes. As a further test of safety and efficacy and to gain additional experience with this vaccine, cattle in the first, second, and third trimester of pregnancy were vaccinated with 5×10^7 pfu of RVF MP12. Fetuses of unvaccinated cows at 3 and 5 months gestation were also inoculated with a similar dose of the vaccine. Vaccinated cows and their fetuses were protected against virulent virus challenge at thirty days post vaccination, whereas unvaccinated cows inoculated with virulent virus became febrile, viremic, and aborted. Those vaccinated cows delivered live, healthy calves which were negative for neutralizing antibodies to RVFV at birth, but quickly acquired colostral immunity which protected them against virulent virus challenge. No fetal losses, which could be directly attributed to the vaccine were observed. Calves born to dams vaccinated during the different stages of pregnancy were seronegative at birth but seroconverted upon ingestion of colostrum. Results of in utero inoculation of fetuses, vaccine virus shedding in the milk of cows vaccinated during lactation, and neonatal calf vaccination and protection studies will also be presented. The results of these studies and tests in non-human primates further support the potential of the mutagen-attenuated strain as a safe and efficacious vaccine for human and veterinary use.

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- 461 ORAL IMMUNIZATION USING LIVE ATTENUATED RIFT VALLEY FEVER VIRUS STRAINS. M.L.M. Pitt and A.O. Anderson, Department of Respiratory and Mucosal Immunity, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701, USA.

Rift Valley Fever virus (RVFV) is a hepatotropic, neurotropic virus that is both arthropod and aerosol transmissible. In addition, ingestion of water containing graded doses of wild-type virus strain ZH-501 yielded dose related mortality in mice. Previous studies using both formalin inactivated and irradiated RVFV vaccine established that both systemic and mucosal immunity are required for complete immunological protection, especially against an aerosol viral challenge where olfactory tract spread results in lethal encephalitis in peripherally immune mice. The feasibility of developing a live-attenuated oral vaccine for RVFV was investigated. Mice were immunized with attenuated virus, strains T1 and MP-12, delivered either directly into the mouth (100 μ l volume) or placed in the drinking water. Control groups were given either saline or killed ZH-501 by the same methods. All the mice were challenged by whole body aerosol exposure to 10LD₅₀ ZH-501 on day 35. One hundred percent of the mice in the control groups died while 80% of those mice immunized with the live attenuated strains survived.

- 462 PROTECTIVE EFFICACY IN RATS OF A FORMALIN-INACTIVATED RIFT VALLEY FEVER VIRUS (RVFV) VACCINE AGAINST AEROSOL CHALLENGE. G.W. Anderson, Jr.,* J.O. Lee, A.O. Anderson, N. Powell, G. Meadors. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD. 21701-5011, U.S.A.

Some natural RVFV infections have been associated with aerosol exposures. Currently, laboratory personnel are receiving the formalin-inactivated, TSI-GSD-200 RVFV vaccine. A 1:40, 80% plaque-reduction neutralization titer (PRNT₈₀) is the suggested post-immunization titer for at-risk personnel. Therefore, we wanted to determine if the adult Wistar-Furth (WF) rat, which is susceptible to both hepatitis and encephalitis, is protected with a low RVFV-neutralizing antibody titer after aerosol exposure. RVFV usually causes a fatal hepatitis in the WF rats prior to day 7 postinoculation by a parenteral route. Disease pathogenesis can be altered by a variety of treatments, so that rats succumb to a necrotizing encephalitis, usually during the 2nd or 3rd week postchallenge. The RVFV vaccine was administered subcutaneously in three doses to adult WF rats on days 0, 7, and 28, the same time course used for at-risk laboratory workers. Six (6) months postimmunization, when the PRNT₈₀ had declined to low or undetectable levels, the rats were challenged with 4.3 log₁₀ plaque-forming units (PFU) of the virulent ZH501 RVFV strain in a nose-only dynamic aerosol apparatus. Ninety-seven per cent of the nonvaccinated control rats (33/34) died with a mean time to death \pm standard deviation (MTD \pm SD) of 3.2 \pm 1.0 days (range 3 - 7 days). In contrast, only 32 per cent of the vaccinated animals (33/103) died with a MTD \pm SD of 6.8 \pm 5.3 days (range 3 - 24 days). Twenty-four (24) vaccinated rats died prior to day 7, while 9 only died after day 7. Ocular sequelae (a new finding in WF rats) and the relationship of the PRNT₈₀ with respect to histological findings of hepatitis and encephalitis will be discussed.

V: VIRUS VACCINES

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T CELL DETERMINANTS ON LASSA VIRUS GLYCOPROTEIN (GP-C).

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Lassa fever is a hemorrhagic disease caused by the arenavirus, Lassa virus (LV). Experimental evidence suggests that immunity to LV is mediated by T cells and that antibody plays no role in recovery from infection. Furthermore, priming of guinea pigs or monkeys with the related arenavirus, lymphocytic choriomeningitis virus (LCMV), will cross-protect animals against lethal challenge with LV. We have found that C3H/HeJ mice are protected against normally lethal LCMV challenge if previously primed with a recombinant vaccinia virus construct (V-LSGP-C) which expresses the cDNA for the Lassa envelope glycoprotein precursor GP-C. Thus, some protective epitopes appear to be located on regions of GP-C which are conserved between the LCMV and LV. To identify epitopes relevant to cross-protection, eleven peptides were synthesized that corresponded to segments of Lassa GP-C and that were predicted to contain T cell recognition sites. Eight of these peptides were from regions of GP-C showing a high degree of homology ($\geq 80\%$) between LV and LCMV and the balance were from sections of GP-C showing low homology between the two viruses (25% to 69%). All of these peptides primed C3H/HeJ mice, to varying degrees, for a secondary peptide-specific response *in vitro*. One peptide also induced proliferation of splenic lymphocytes from mice primed with LCMV or V-LSGP-C indicating that it represents a virus-specific T cell determinant. This finding appears to be the first molecular definition of a T cell determinant common to both of these arenaviruses. The relevance of this determinant to cross-protection is under investigation.

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Neutralizing Antibody Following Formalin Inactivated Hepatitis A Vaccine: Persistence of Antibody and Neutralization of Isolates from three Continents.

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We have previously reported the development of neutralizing antibody in volunteers given 4 doses of inactivated hepatitis A virus (HAV) vaccine produced from infected MRC-5 cells (Viral Hepatitis and Liver Disease, A.J. Zuckerman, Ed. 1988, p 94-96). Further studies were carried out to evaluate the ability of vaccine-induced-antibody to neutralize five isolates from three continents and to determine the persistence of neutralizing antibody. The radioimmune focus inhibition test was used to measure neutralizing antibody. Two months after the fourth dose, serum from each volunteer neutralized HAV isolates from Kansas, Alaska, Germany, North Africa and Panama. With two exceptions, the titers obtained using test viruses were within a single four-fold dilution of the titers obtained using the vaccine strain (HM 175), which was from Australia. In one instance the titer was 16-fold higher, the other 16-fold lower. At both 12 and 18 months after the fourth dose, the serum specimens neutralized the HM-175 homologous virus. Although the geometric mean titer fell from 1:269 two months after the fourth dose to 1:48 at 18 months, the serum from each volunteer continued to neutralize the virus. These findings indicate that this inactivated vaccine should protect against HAV strains of widely diverse geographic origin. Furthermore the vaccine induced a prolonged neutralizing antibody titer which greatly exceeded the titer associated with administration of immune serum globulin.

W: KINETOPLASTIDIA - BIOCHEMISTRY,
MOLECULAR BIOLOGY AND CHEMOTHERAPY

465 Characterization of an RNA virus from the parasite
Leishmania.

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Medical School, and Harvard School of Public Health, Boston, MA.

We were interested in screening a series of isolates from the protozoan Leishmania for the presence of viruses. The experimental procedure we used was based on an enzymatic assay originally developed for viral RNA-dependent RNA polymerases. Simultaneously, total promastigote nucleic acid preparations were analyzed for the presence of viral genome and/or transcripts. Two isolates, both classified as L. braziliensis guyanensis, were found to be positive for RNA polymerase activity and to carry a large (6 Kilobases) RNA species. The polymerase reaction products hybridized to the 6 kb RNA, believed to be the viral genome. In conjunction with electron microscopic observations these results indicate the presence of an RNA virus in these Leishmania isolates. Preliminary evidence suggests that in our RNA dependent RNA polymerase assay we are making primarily plus sense RNA. (Widmer et al, PNAS *in Press*, 1989)

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**MONOCLONAL ANTIBODIES AGAINST *T. cruzi* NEURAMINIDASE REVEALS
ENZYME POLYMORPHISM AMONG DIFFERENT STRAINS, RECOGNIZE A
SUBSET OF PARASITES AND ENHANCE INFECTION *IN VITRO*. *R. P. Prioli and
M.E.A. Pereira. New England Medical Center Hospitals, 750 Washington Street, Box
041, Boston, Mass, 02111.**

Two murine monoclonal antibodies (TCN-1 and TCN-2) were produced against *T. cruzi* neuraminidase and used to analyse some of the molecular and biological properties of the enzyme. Antibody selection was performed based on several criteria which included the ability to inhibit and immunoabsorb enzyme activity and recognition and immunoprecipitation of neuraminidase from parasite lysate or from supernatant containing material released by the parasite. The antigens recognized by the monoclonal antibodies were stage specific being present in tissue culture trypomastigotes but not in the amastigote, epimastigote or metacyclic trypomastigote forms. Immunoblot analysis using TCN-1 and TCN-2 revealed a molecular polymorphism among the different strains of *T. cruzi*. The neuraminidase from Silvio X-10/4, which displayed the simplest molecular pattern, was further characterized by two dimensional gel electrophoresis. Immunofluorescence and complement mediated lysis assays showed that the monoclonal antibodies identified a subpopulation of neuraminidase positive parasites. *In vitro* infection of host cells by trypomastigotes was enhanced in the presence of antibody concentrations capable of inhibiting neuraminidase activity. These experiments support our hypothesis that neuraminidase or neuraminidase positive parasites down-regulate *T. cruzi* infection.

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MOLECULAR BIOLOGY AND CHEMOTHERAPY

467 INHIBITION OF ANTIMONY-RESISTANT LEISHMANIA WITH A BIS(BENZYL)
POLYAMINE ANALOG

*R.J. Baumann, A.J. Bitonti, P.P. McCann, W.L. Hanson and A. Sjoerdsma
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Georgia, Athens, Georgia.

We previously reported that a bis(benzyl) polyamine analog, (MDL 27695, N,N'-bis[3-[(phenylmethyl)amino]propyl]-1,7-diaminoheptane) possessed potent antimalarial activity toward Plasmodium falciparum in vitro and Plasmodium berghei in vivo (1). We now show that MDL 27695 has antileishmanial activity against Leishmania donovani amastigotes in vitro and in vivo. MDL 27695 was initially tested in L. donovani infected monolayers of elicited peritoneal macrophages from BALB/c mice. Amastigote growth was completely inhibited by 1 μ M MDL 27695 (IC_{50} = 1 nM) and spermine did not reverse this inhibition. BALB/c mice and Syrian golden hamsters infected with L. donovani were treated with MDL 27695. Administration of 15 mg/kg, three times per day for 5 days in mice, and 5 mg/kg, three times per day for 4 days in hamsters, suppressed liver parasite burdens 96% and 74%, respectively. The ED_{50} was 2.3 mg/kg in mice and about 1 mg/kg in hamsters. In hamsters, MDL 27695 was also found to be equally effective against antimonial-sensitive and antimonial-resistant L. donovani, suggesting a different mechanism of action for the two types of drugs. Coadministration of N¹,N⁴-bis(butadienyl)-butanediamine (MDL 72527) to mice to inhibit polyamine oxidase in vivo did not affect the antileishmanial activity of MDL 27695. Thus, the mechanism of action of MDL 27695 does not appear to be related to polyamine biosynthesis or to a requirement for its oxidation to toxic metabolites, but may involve interference with DNA and RNA synthesis as previously found in P. falciparum (1), possibly due to displacement of natural polyamines.

1) Bitonti et al. (1989) Proc. Natl. Acad. Sci. U.S.A., 86:651-655.

468 EFFECTS OF ETHER ANALOGUES OF LYSOPHOSPHOLIPIDS ON LEISHMANIA.

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Parasitic infection induces inflammation which results in chemotaxis and activation of phagocytic cells. The inflamed lesions of normal and parasitized tissues release decomposed products of membranous lipids, lysophospholipids and alkylglycerols, each of which is a potent macrophage activating agent. The purpose of the study was to determine whether ether analogues of lysophospholipids were leishmanicidal and whether macrophages activated by these agents were also able to kill intracellular Leishmania. When promastigotes (1.8×10^6 /ml) of Leishmania major and Leishmania donovani were treated with 1.5-3.0 μ g Rac-1-octadecyl-2-methyl-glycero-3-phosphocholine (ET-18-OCH₃-choline) or rac-SN-1-(3)-dodecyl-glycerol (DDG)/ml, a 100% leishmanicidal activity was observed within 48-72 hr of treatment as determined by microscopic quantification using trypan blue exclusion test. At a concentration of 2.0 μ g/ml, ET-18-OCH₃-choline produced a 100% leishmanicidal activity within 24 hr as compared with 50% leishmanicidal activity of DDG. Furthermore, macrophages from mice treated with these agents three days previously, had a significantly reduced number of intracellular amastigotes. As reported previously, at three days post-treatment with these agents, macrophages are activated and their phagocytic and cytotoxic activities are greatly enhanced. Such activated macrophages showed efficiently enhance leishmanicidal activity. Therefore, these lipid metabolites have dual beneficial effects for the host by enhancing macrophage leishmanicidal activity and direct cytotoxic activity.

W: KINETOPLASTIDIA - BIOCHEMISTRY,
MOLECULAR BIOLOGY AND CHEMOTHERAPY

- 469 METABOLIC PREADAPTATION OF METACYCLIC AFRICAN TRYPANOSOMES FOR
THE MAMMALIAN BLOODSTREAM J. Kiara¹, W.R. Fish², AND E.J. Bienen²

¹Dept. Biochemistry, University of Nairobi and

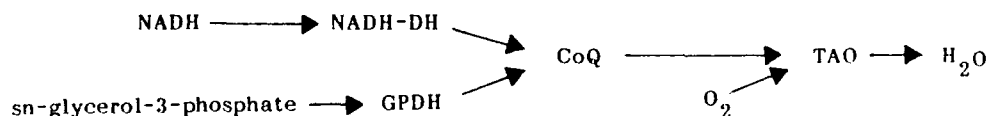
²International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

It has previously been reported that respiratory metabolism of in vitro derived metacyclic trypomastigotes of Trypanosoma congolense is similar to bloodstream forms with the trypanosome alternative oxidase as the primary terminal oxidase and glucose as the preferred substrate. We now report on some glycosomal and mitochondrial enzyme levels of this stage of the life cycle. Glycolytic enzyme activities of T. congolense metacyclics are similar to those found in bloodstream forms with elevated levels of most enzymes including the putative rate-limiting enzyme hexokinase. Phosphoglycerate kinase and malate dehydrogenase, two enzymes showing differentially compartmentalized activity during the life cycle, were also studied. Preliminary evidence indicates that metacyclics have these enzyme activities localized predominantly within the glycosome as in bloodstream forms. Additionally, the mitochondrial succinate dehydrogenase and succinate cytochrome c reductase complex are also present in metacyclics but have reduced activity. Our data suggest that metacyclics are almost completely adapted metabolically for life in the mammalian bloodstream.

- MITOCHONDRIAL PROTON MOTIVE FORCE IN BLOODSTREAM AFRICAN
470 TRYPANOSOMES DEMONSTRATED BY RHODAMINE 123. M. Saric^{1*}, E.J. Bienen², G. Pollakis¹, R.W. Grady³, and A.B. Clarkson, Jr.¹

¹New York University Medical Center, New York, NY, 10016; ²ILRAD, Nairobi, Kenya; ³Cornell University Medical Center, New York, NY, 10021.

The vital stain rhodamine 123 (rh-123) selectively accumulates in the mitochondrion of intermediate and short-stumpy forms of Trypanosoma brucei. Sensitivity to 2,4-dinitrophenol demonstrates that this is due to a proton gradient. Abrogation by rotenone indicates that the NADH-ubiquinone reductase complex is active and responsible for the proton gradient. The possibility that cytoplasmic ATP and the mitochondrial F₁F₀ ATPase could have generated the gradient was eliminated by the observation that oligomycin, an inhibitor of F₁F₀ ATPase, did not block rh-123 accumulation. Because neither antimycin A nor cyanide blocked rh-123 accumulation and bloodstream trypanosomes are known to contain no cytochromes, electrons must be transferred from the ubiquinone pool by a non-cytochrome mediated system. Rh-123 accumulation is sensitive to salicylhydroxamic acid, an inhibitor of the trypanosome alternative oxidase (TAO), therefore we conclude that electrons from the NADH-ubiquinone reductase complex are transferred to the ubiquinone pool and from there to molecular oxygen by the TAO. The mitochondrial electron transport system of intermediate and short stumpy forms can be diagramed as follows:



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MOLECULAR BIOLOGY AND CHEMOTHERAPY

- 471 INVOLVEMENT OF HUMAN TRANSFERRIN IN THE TRANSFER OF IRON TO
TRYPANOSOMA CRUZI.
M.F. Lima* and F. Villalta, Division of Biomedical Sciences,
Meharry Medical College, Nashville, TN.

The basic mechanisms by which the obligate intracellular human protozoan Trypanosoma cruzi takes up micronutrients from the host to multiply in mammalian cells are not known. An understanding of these processes may facilitate the formulation of strategies for blocking the mammalian phase of the parasite cycle. We have been investigating the consequences of the interaction of host iron-binding proteins with amastigote forms of the parasite. We have found that amastigotes present receptors for human transferrin and that these receptors are developmentally regulated. Iron is required for amastigote growth in cell-free medium. We have also found that amastigote growth in cell-free medium is inhibited when parasites are grown in media supplemented with ferro-transferrin depleted serum. Addition of ferro-transferrin but not apotransferrin restored parasite growth. ⁵⁹Fe-transferrin bound to amastigotes at 4°C for 1 hour was readily dissociated from the parasite surface upon mild acid treatment. However, this treatment did not disrupt binding that occurred at 37°C, indicating that transferrin had obtained an intracellular location. Western blots of solubilized amastigote membranes probed with anti-human transferrin receptor IgG indicate that a protein of molecular weight 200kDa, which is present on the amastigote cell surface, interacts with transferrin. These results indicate that iron appears to be delivered to T. cruzi amastigotes by transferrin receptor mediated endocytosis. (Supported by USAID grant DAN-5053-G-SS-8052-00)

- 472 IDENTIFICATION OF A GENE WHICH IS DIFFERENTIALLY EXPRESSED DURING DEVELOPMENT OF
TRYPANOSOMA RHODESIENSE FROM BLOODSTREAM TO PROCYCLIC TRYPOMASTIGOTES
L.E. Wirtz, D.A. Sylvester, and *G.C. Hill. Meharry Medical College, Nashville, TN.

Differentiation of bloodstream trypomastigotes of Trypanosoma rhodesiense to procyclic forms is induced by introduction into culture at 27°C. We sought to identify genes whose expression is altered upon this environmental shift and which are therefore implicated in either the activation or execution of the new developmental program. We report here on the molecular cloning of one such developmentally regulated gene from T. rhodesiense and on features of its expression during differentiation. In Northern analyses, insert from this clone detects a 1.6 kb p[A+] transcript which is ten to thirty fold more abundant in established procyclics than in bloodstream forms. The kinetics with which this transcript accumulates in steady-state RNA during differentiation were examined by probing Northern of total RNA isolated from transforming organisms at different timepoints after introduction into culture. An increase above blood stage levels is observed within 0.5 hours of the environmental shift. Steady-state levels peak at 50 fold bloodstream levels about 24 hours before 50% of the population have acquired a procyclic morphology. The transcript fails to accumulate in the absence of protein synthesis, pointing to a requirement for the *de novo* synthesis of regulatory factors following the shift. The transcript is also detected and shows similar regulation in T. brucei strains. In addition, we have identified a nontransforming T. brucei strain in which transcripts remain at the bloodstream level for greater than 24 hours after introduction into culture, until viability is lost, suggesting that this variant is blocked early in the developmental program. Preliminary DNA sequence data reveal no significant homology to known proteins or genes. A more complete dissection of the role of this gene in differentiation including characterization of the gene product and of its level of regulation is underway and should contribute to our understanding of what regulatory mechanisms come into play to orchestrate transitions between developmental stages as these parasites differentiate during their life cycle.

Supported by NIH grant AI-21159.

W: KINETOPLASTIDIA - BIOCHEMISTRY,
MOLECULAR BIOLOGY AND CHEMOTHERAPY

- 473 DNA METHYLATION PATTERNS OF *TRYPANOSOMA BRUCEI BRUCEI* DURING DFMO INDUCED TRANSFORMATION IN VIVO. B.F. Giffin* and S.J. Wunderle. Department of Biology, University of Dayton, Dayton, OH.

In order to determine whether the DFMO induced transformation of the long slender (LS) trypanosome to the short stumpy form (SS) is accompanied by DNA demethylation, restriction digests were conducted using a series of restriction enzymes that will not cut GC recognition sequences when cytosine is methylated. DNA samples were prepared from LS and DFMO induced intermediate (INT) and SS trypanosomes and restricted with HpaII and MspI. These enzymes, both of which cut the sequence 5'-CCGG-3', can be blocked by cytosine methylation. MspI will not cleave ^{me}CCGG and HpaII will not cleave C^{me}CGG. Agarose gel electrophoresis following restriction indicated that DNA from LS trypanosomes was cut by HpaII but not by MspI, whereas INT and SS trypanosome DNA was cut by both restriction enzymes. When the DNA from all three morphological forms was incubated with HpaII methylase and S-adenosylmethionine followed by restriction with HpaII, the DNA was protected from restriction. Other restriction enzymes (ApaI, HaeIII, HhaI, SmaI) with GC recognition sequences that require the absence of methylation for cutting showed no differences in methylation states between transformed and nontransformed trypanosomes. It can be concluded that the recognition sequence CCGG is methylated at the 5' cytosine in the DNA of the LS trypanosome but not in the DNA of the DFMO induced INT or SS forms. In contrast, the internal cytosine is not methylated in any of these bloodstream forms. These observations suggest the possibility that demethylation of certain DNA sequences following polyamine inhibition by DFMO may be one regulatory mechanism for the activation of transformation specific genes.

- 474 DIFFERENTIAL SENSITIVITY OF *TRYPANOSOMA B. RHODESIENSE* CLINICAL ISOLATES TO DIFLUOROMETHYLORNITHINE AND ARSENICALS. C.J. Bacchi^{1*}, H. Nathan¹, N. Yarlott¹, P. Sayer², A. Njogu², P.P. McCann³, A.J. Bitonti³ and A.B. Clarkson, Jr.⁴ ¹Haskins Labs and Biology Dept., Pace University, New York, NY 10038. ²Kenya Trypanosomiasis Research Institute (KETRI), Muguga, Kenya. ³Merrell Dow Research Institute, Cincinnati, OH 45215. ⁴Dept. of Medical and Molecular Parasitology, New York University Medical Center, New York, NY 10010

Sixteen clinical isolates from the KETRI (formerly EATRO) strain bank were studied for sensitivity to DL- α -difluoromethylornithine (DFMO) and arsenicals (AS). Using these strains as acute infections in mice and *T. b. brucei* Lab 110 EATRO as sensitive strain, we found 7 DFMO-refractory & 3 AS-refractory strains. All strains refractory to AS were also refractory to DFMO. Ornithine decarboxylase (ODC) activity was 40-60 nmoles/mg protein/h for all strains. DFMO time-dependent inhibition kinetics (1) on crude ODC preparations produced K_i values of 25-50 μ M. [³H] DFMO uptake by intact trypanosomes in vitro ranged from 500-700 pmoles/mg protein/h except for 1 refractory strain whose uptake was only 200 pmoles/mg protein/h.

AS resistance was examined by spectrophotometrically following lysis of trypanosomes suspended in serum (2) with melarsen oxide. The AS levels required for 50% lysis of sensitive strains in 30 min was 2.5 to 15 μ M, but 150-200 μ M for refractory strains. AS action is based on depletion of trypanothione, a unique polyamine-containing thiol, by the formation of a complex between AS and trypanothione (3). Total free thiol levels were therefore measured and these were found to be 4-5 times higher in the AS-refractory strains.

1. Bitonti, A.J. et al. 1985. Biochem. Pharmacol. 34:1773; 2. Clarkson, A.B. & B.O. Amole. 1982. Science 216:132; 3. Fairlamb, A. et al. 1989. PNAS (USA) 86:2607. Funded by NIH AI17340, UNDP/World Bank/WHO 890064; and USAID project 86-06047.

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IDENTIFICATION AND CHARACTERIZATION OF GLYCOSYLPHOSPHATIDYLINOSITOL-LINKED SCHISTOSOMA MANSONI ADULT WORM IMMUNOGENS. *Samir Y. Sauma and Mette Strand. Johns Hopkins University School of Medicine, Department of Pharmacology and Molecular Sciences, Baltimore, MD 21205

Metabolic radiolabeling of adult worms of Schistosoma mansoni with [³H]myristic acid has revealed that the fatty acid is incorporated into more than 20 proteins. We report here that two of these proteins, a 200-kDa glycoprotein previously shown to be exposed on the surface of the adult worm following praziquantel treatment and a 22-kDa glycoprotein that shows an enhanced immune reactivity with sera of vaccinated mice, are anchored to the adult worm membrane via a glycosylphosphatidylinositol (GPI) linkage. Both antigens partitioned preferentially into the detergent phase of Triton X-114 and were susceptible, following immunoaffinity purification, to hydrolysis by PI-specific phospholipase C (PIPLC) from Bacillus thuringiensis and phospholipase C from Bacillus cereus. Diacylglycerol (DAG) was released following hydrolysis by bacterial PIPLC; however, Trypanosoma brucei GPIPLC failed to release the diacylglycerol from either protein. Treatment with nitrous acid generated phosphatidylinositol (PI), and phospholipase D from rat serum cleaved phosphatidic acid from both proteins. Although the functional significance of these GPI-anchored proteins is unknown, their release from the surface of schistosome may contribute to immune evasion.

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EVIDENCE OF CROSS-REACTIVE, SHARED IDIOTYPES ON ANTI-SEA ANTIBODIES FROM HUMANS AND MICE WITH SCHISTOSOMIASIS. *M.A. Montesano, G.L. Freeman, G. Gazzinelli and D.G. Colley. Univ. Fed. Juiz de Fora, Juiz de Fora, MG, Brazil and VA Med. Ctr. and Vanderbilt Univ. Sch. Med., Nashville, TN.

We have shown that some mouse and human anti-SEA monoclonal antibodies (MAbs) stimulate lymphocytes (PBMC) from patients. AM1 and AM5 [anti-SEA Abs from 2 serum pools from chronic infected patients] stimulated proliferation of spleen cells from mice infected for 8 or 16 weeks. However AM8 (anti-SEA Abs from hepatosplenic patients) failed to stimulate mouse cells. In earlier studies AM1 and AM5 Ids stimulated human PBMC, while AM8 Ids did not. Using AM1-specific, AM5-specific, AM8-specific or human anti-SEA MAb E5-specific rabbit antisera in competitive ELISA systems, sera from mice 8 and 16 weeks after infection competed with AM1, AM5 or E5, but not AM8, indicating that some of the Abs in sera from infected mice bear Ids which are expressed on the human Abs in AM1 and AM5, and E5, but not Abs in AM8. Sera from 8 week-infected mice inhibited the most against AM1, AM5 and E5, and they were more stimulatory for either human PBMC of mouse spleen cells, than those from later in infection. We conclude that anti-SEA Abs from humans with chronic schistosomiasis and mice with 8 week Schistosoma mansoni infections, share significant levels of cross-reactive Ids. (Supported by AI-26505, CNPq and Veterans Administration.)

X: SCHISTOSOMIASIS - ANTIGENS AND IMMUNOGENS

477 CHARACTERIZATION OF TRIOSE PHOSPHATE ISOMERASE cDNA
AND GENOMIC CLONES FROM SCHISTOSOMA MANSONI.

*M.G. Reis, A. Gross, D. Harn and C. Shoemaker.

Department of Tropical Public Health, Harvard School of Public Health.

The 28 kDa antigen of S. mansoni has been identified as triose phosphate isomerase (TPI) and an anti-28 kDa monoclonal antibody has been shown to confer partial protection to S. mansoni infection in mice. We have cloned and characterized a full-length cDNA for S. mansoni TPI and find that approximately 50% of the encoded amino acids are identical or closely related between S. mansoni and all procaryotic and eukaryotic TPI proteins previously reported. Only about 25% of the amino acids differ substantially from mammalian TPI. In addition, we have obtained genomic clones containing all of the S. mansoni TPI coding exons and substantial additional 5' and 3' sequence. DNA sequencing of the S. mansoni TPI gene revealed the existence of six exons. The location of all five introns are precisely conserved relative to those identified in mammalian TPI genes. Introns contain standard splice donor and acceptor sites although, in most cases, they are much larger than in mammals. The first intron, though, is only 42 bp in length. A putative promotor element has also been identified.

478 PEPTIDE FRAGMENTS OF RECOMBINANT SCHISTOSOME TRIOSE PHOSPHATE
ISOMERASE RECOGNIZED BY ANTI-28 Kda ANTIBODIES.

*D.A. Harn, and W. Gu. Department of Tropical Public Health.
Harvard School of Public Health, Boston, MA.

Previously we reported that a protective monoclonal antibody recognized a 28 kDa antigen present in all stages of schistosomes. Amino acid sequence analysis of tryptic peptides followed by sequence homology analysis showed that this 28 kDa antigen was the glycolytic and gluconeogenic enzyme, triose phosphate isomerase.

The native schistosome antigen was found to function as TPI in an enzymatic assay. Subsequently, both monoclonal antibody and rabbit anti-28 kDa antibodies were shown to completely inhibit the enzymatic activity. Both antibodies were shown to be specific for schistosome TPI as they did not bind to nor inhibit the activity of human, rabbit, yeast or dog TPI's.

Using a probe constructed from human TPI, recombinant schistosome TPI was cloned. The complete coding sequence was inserted within an E. coli expression vector and encodes a 28 Kda protein when produced in E. coli. The protein retains the enzymatic and immunologic properties of native TPI, including inhibition by the anti-TPI antibodies.

We have used staphylococcal V8 protease and cyanogen bromide to generate peptide fragments. Cyanogen bromide fragmentation resulted in the generation of an antigen/enzyme fragment of between 12-15 kDa which is recognized by the rabbit anti-28 kDa antibody. With SV8 protease, several smaller molecular weight peptides also appear to be antigenic. Sequence analysis of these peptides may allow for the generation of a model which allows us to compare the immunogenic sites in relation to the active site on the molecule and thus, determine whether or not both antibodies could be used as models for anti-TPI drug design.

X: SCHISTOSOMIASIS - ANTIGENS AND IMMUNOGENS

479 SCHISTOSOMA MANSONI EGF RECEPTOR HOMOLOGUE: CLONING AND CHARACTERIZATION. *C.B. Shoemaker, M.R. Reis, A. Landa, and L. Stein. Dept. of Tropical Public Health, Harvard School of Public Health, Boston Mass.

We have begun an effort to clone the coding DNA for several S. mansoni membrane receptors based on homology to analogous receptors from higher eukaryotes. The goal is to express these receptors in mammalian host cells for use as models in a study to investigate the potential of schistosomal membrane proteins as vaccine candidates. One receptor that was chosen as a cloning target was the schistosome homologue of the epidermal growth factor (EGF) receptor. We screened an S. mansoni cDNA library at low stringency with a probe prepared from the chicken erb-b tyrosine kinase domain and obtained initial positive clones. These clones were used as probes to obtain a complete copy of the coding DNA for a protein with substantial homology to the EGF receptor class of molecules. DNA sequence of the clones predicts a 200 kd translation product that, like the other known EGF receptor homologues, contains a secretory leader sequence, a cysteine-rich extracellular domain, a hydrophobic transmembrane sequence, a tyrosine kinase domain and a proline-rich COOH-terminus. The extracellular domain of the S. mansoni EGF receptor homologue (SER) is only distantly related to mammalian EGF receptors and appears unlikely to respond to mammalian growth factors. The tyrosine kinase domain of SER is the most highly conserved region (45% to mammalian) and contains virtually all the residues that are conserved within the sarc family of tyrosine kinases. While screening S. mansoni cDNA libraries with SER probes, we have identified and characterized numerous variant clones that encode a portion of the amino-terminal coding region but subsequently diverge. The corresponding RNAs would encode truncated SER molecules, several that should be secreted and one that should remain in the membrane. The origin, function and abundance of these transcripts is currently being investigated.

480 AUGMENTED HUMAN ANTIBODY RESPONSE TO SMW68, A CANDIDATE S. MANSONI VACCINE, IS ASSOCIATED WITH PROTECTION AGAINST REINFECTION FOLLOWING THERAPY. *C.H. King, M. Amer, A. El Hawey, and A.A.F. Mahmoud. Al-Azhar University, Cairo, Egypt, and Case Western Reserve University, Cleveland, OH.

Vaccine-induced antibody response to the S. mansoni surface antigen SmW 68 is associated with protective immunity to experimental infection in mice. Further, in chronically-infected humans, seroepidemiological studies have shown a significant inverse correlation between levels of circulating anti-SmW68 antibody and intensity of infection. To test whether therapy-induced changes in antibody response to SmW68 are predictive of infection status after praziquantel treatment, we evaluated anti-SmW68 and anti-crude antigen (SWAP) antibody levels in 50 children aged 9-15 having light or moderate S. mansoni infection (75-300 eggs/gm of stool). In this group, pretreatment anti-SmW68 antibody levels (determined by direct ELISA) were significantly higher in those with lower levels of infection ($R = -0.6423$, $P < 10^{-4}$). These pretreatment antibody levels were not, however, predictive of infection status at 1-12 mos after therapy. In contrast, it was noted that individuals who showed an increase in anti-SmW68 antibody level after therapy had significantly lower prevalence of infection at 6 and 12 mos after therapy: at 6 mos, prevalence was 5% for those with increased levels ($N=37$) vs. 92% for those with decreased levels ($N=13$) ($P < 10^{-4}$). Respective values at 12 mos were 27% and 92% ($P < 0.0002$). These findings were not observed for antibody response to SWAP. We conclude that an elevated antibody response to the candidate schistosome vaccine SmW68 following therapy is associated with reduced risk of reinfection, and that established or augmented humoral immunity to SmW68 may play a significant role in protective immunity.

X: SCHISTOSOMIASIS - ANTIGENS AND IMMUNOGENS

- 481 IDENTIFICATION OF A DOMINANT EPITOPE ON THE CYSTEINE PROTEINASE FROM *S. MANSONI* ADULT WORMS. F. Monroy, M.H. Dresden, and C.L. CHAPPELL* Verna and Marrs McLean Department of Biochemistry and the Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas.

A cysteine proteinase (CP1) from adult *S. mansoni* is an important component of host hemoglobin degradation in the parasite gut. This enzyme is released into host circulation and is highly immunogenic in human and animal infections. CP1 has been purified (Chappell and Dresden, 1987), characterized and cloned (Davis et al., 1987). Hydrophilicity analysis of the primary sequence was done, and the three highest peaks (putative antigenic sites) were examined. The sequences of all three sites were strikingly similar. Sites I and II, which were separated by only three residues, were 87% homologous and were synthesized as a single 22-mer peptide (SMP 22). Additionally, a 12-mer peptide (SMP 12) containing site II only, was also synthesized. The antigenicity of the peptides was analyzed by direct and competition ELISA using sera from mice and humans infected with *S. mansoni*. Both peptides were covalently bound to microtiter wells and were recognized by IgG from *S. mansoni*-infected sera, but were negative with matching, non-infected sera. Furthermore, these peptides, pre-incubated with infected sera from both species, blocked IgG reactivity to the intact protein. These results indicate that the SMP 12 ("site II" peptide) is sufficient to block IgG binding to intact protein. Antibody to site II may also bind to sites I and III by virtue of their sequence similarities. Thus, the SMP 12 and SMP 22 peptides may be useful and cost-effective immunodiagnostic probes that can be adapted to field-compatible assay systems.

- SCHISTOSOMA MANSONI: THE ROLE OF CALCIUM IN THE REGULATION OF CERCARIAL PROTEINASE RELEASE. A.C. Fusco and B. Salafsky. University of Illinois College of Medicine at Rockford, Rockford, IL.

The secretion of proteinase by cercariae is a crucial step in penetrating the host skin barrier. This release can be stimulated by essential fatty acids and our laboratory has postulated that eicosanoids may be involved in its regulation. Since Ca^{2+} plays an important role in the stimulation of eicosanoid release in many cell types and Ca^{2+} is also important in cercarial proteinase function, we examined the regulatory role of Ca^{2+} in cercarial proteinase secretion. The method of McKerrow *et al.* (Lab. Invest. 49:195, 1983) was used to quantitate cercarial proteinase release by the degradation of a ^3H labeled extra-cellular matrix coated with $4 \mu\text{g}/\text{cm}^2$ linoleate. Cercariae incubated in Ca^{2+} free media did not have significant proteinase activity; however, the Ca^{2+} channel blockers nifedipine, verapamil and fendiline were unable to inhibit cercarial proteinase release. Ca^{2+} ionophore ($1 \mu\text{g}/\text{cm}^2$) was as potent as linoleate in stimulating proteinase release and this effect was inhibited by esculetin, a lipooxygenase inhibitor; however, this effect was not inhibited by removal of Ca^{2+} from the incubation media. In addition, neither PGE_2 (0.01 to $100 \mu\text{g}/\text{cm}^2$) nor LTB_4 (0.0001 to $1 \mu\text{g}/\text{cm}^2$) alone were able to stimulate proteinase release. These preliminary data, seem to indicate a regulatory role for internal Ca^{2+} stores released via cercarial eicosanoid synthesis. This release may not involve Ca^{2+} channels, nor can it be stimulated by external application of eicosanoids.

ANNUAL FRED SOPER LECTURE

No Abstract Available

- 483 IN SEARCH OF A NATIONAL AGENDA FOR INTERNATIONAL HEALTH PROBLEMS.
W.H. Foege. Carter Center, Atlanta, GA.

SYMPOSIUM: HANSEN'S DISEASE: ADVANCES IN CLINICAL AND
EXPERIMENTAL RESEARCH

(No abstracts available)

Hawaii is singularly appropriate for a symposium on Hansen's Disease. The Hawaiian Islands have had a unique and important experience with Hansen's Disease, or leprosy, beginning with a major epidemic after its introduction to the native population in the early 1800's. The incidence and prevalence rates for leprosy in Hawaii are still among the highest in the United States, largely due to importation of cases by immigration from Asian and Pacific countries. In 1873, the same year that Hansen discovered the leprosy bacillus, Fr. Damien de Yeuster arrived on the island of Molokai to begin his exemplary work among patients afflicted with leprosy, and isolated because of it. This year marks the 100th anniversary of Damien's death, being commemorated by a number of educational events in Hawaii and his native Belgium.

This symposium exploring recent advances and new directions in research into this infection, by scientists from several countries, comes appropriately as a conclusion to this year of historical recollection. The unique immunopathologic spectrum of host response to infection with M. leprae provides one of the most intriguing models of cellular immunity in man. In the last two decades, major advances in immunology, genetics, and molecular biology have opened many avenues for research into the mechanisms operating in infection with M. leprae, but the mystery of this unique host-pathogen interaction remains essentially unsolved.

This symposium presents a fresh examination of new approaches and evidence from selected areas of both clinical and experimental research now in progress, crossing customary disciplinary lines. Presentations will highlight recent advances in the understanding of genetic influences on host response to M. leprae, immunology of human leprosy lesions in situ, primate models of infection, serology, epidemiology, and molecular approaches to the possible development of a leprosy vaccine.

- 484 GENETIC RESISTANCE TO INTRACELLULAR INFECTION. E. Skamene. Center for the Study of Host Resistance, McGill University, Montreal, CANADA.
- 485 GENETIC DETERMINANTS OF SPECIFIC IMMUNITY TO M. LEPRAE. R.R.P. de Vries. University Hospital, Leiden, THE NETHERLANDS.
- 486 INSIDE THE SKIN: THE LOCAL IMMUNE AND INFLAMMATORY MILIEU IN LEPROSY. D.M. Scollard. John A. Burns School of Medicine, University of Hawaii, Honolulu, HI.

SYMPOSIUM: HANSEN'S DISEASE: ADVANCES IN CLINICAL AND
EXPERIMENTAL RESEARCH (Continued)

- 487 LEPROSY IN NON-HUMAN PRIMATES -- NATURALLY ACQUIRED AND EXPERIMENTAL
 INFECTIONS. W.M. Meyers, President, International Leprosy
 Association; Mycobacteriology Section, Armed Forces Institute of
 Pathology, Washington, DC.
- 488 EPIDEMIOLOGY OF LEPROSY: THE CURRENT EPIDEMIC IN MICRONESIA.
 R. Worth and M. O'Leary. Hawaii State Health, Honolulu, HI;
 and Pohnpei, EASTERN CAROLINE ISLANDS.
- 489 SEROLOGY OF LEPROSY. J. Douglas. University of Hawaii, Honolulu, HI.
- 490 MOLECULAR IMMUNOLOGY OF NEW GENERATION VACCINES FOR HANSEN'S DISEASE.
 J.D. Watson. University of Auckland, NEW ZEALAND.

SYMPOSIUM: MALARIA IN PREGNANCY

- 491 INTRODUCTION. R.S. Desowitz. University of Hawaii, Honolulu, HI.
- 492 EPIDEMIOLOGY OF MALARIA IN PREGNANCY IN AFRICA. R. Steketee. Centers
 for Disease Control, Atlanta, GA.
- 493 EPIDEMIOLOGY OF MALARIA IN PREGNANCY IN SOUTHEAST ASIA AND THE
 PACIFIC. G. Buchbinder. University of Hawaii, Honolulu, HI.
- 494 PLACENTAL PATHOLOGY IN HUMAN MALARIA. M. Aikawa. Case Western Reserve
 University, Cleveland, OH.
- 495 ANIMAL MODELS OF PREGNANCY-RELATED ENHANCED MALARIA. R. Desowitz.
 University of Hawaii, Honolulu, HI.
- 496 THE IMMUNE RESPONSE IN PREGNANCY-RELATED ENHANCED MALARIA. W. Eling.
 Catholic University, School of Medicine, Nijmegen, THE NETHERLANDS.
- 497 MATERNAL TRANSFER OF PROTECTIVE ANTIBODIES IN MALARIA. M.
 Hollingdale. Biomedical Research Institute, Rockville, MD.
- 498 TREATMENT AND PREVENTION OF MALARIA IN PREGNANCY. J. Breman. Centers
 for Disease Control, Atlanta, GA.
- 499 MATERNAL AND FETAL IMMUNE PHENOMENA ASSOCIATED WITH HUMAN PREGNANCY.
 A. Beer. The Chicago Medical School, Chicago, IL.
- GENERAL DISCUSSION.
- 500 CLOSING REMARKS. M. Aikawa. Case Western Reserve University,
 Cleveland, OH.

Y: RETROVIRAL INFECTIONS

- 501 CLINICAL AND ENDOSCOPIC EVALUATION OF DIARRHEA AND WASTING IN KENYAN PATIENTS INFECTED WITH HIV. R.T. Bryan*, R.L. Owen, A. Cali, F.A. Okoth, J.B.O. Were, F. Sang, H.C. Spencer. Parasitic Diseases Branch, CDC, Atlanta, GA.; University of California, San Francisco, CA; Rutgers University, Newark, NJ; Kenya Medical Research Institute, Nairobi, Kenya.

Chronic wasting, with or without chronic diarrhea, may be the most common manifestation of infection with human immunodeficiency virus (HIV) in Africa; very few detailed clinical investigations of HIV-infected African patients with diarrhea and/or wasting (D/W) have been reported. We identified 12 HIV-positive Kenyan patients with D/W and enrolled them for intensive clinical evaluation. Physical examinations, routine blood tests, urinalyses, stool cultures, stool examinations for viral and parasitologic pathogens, and esophagogastroduodenoscopy with small bowel biopsies were performed for each patient. Ten non-HIV-infected patient-volunteers were enrolled as controls to undergo the same clinical evaluation. The most clinically significant results were obtained from coproparasitologic specimens and small bowel tissue. Pathogenic intestinal parasites were identified in 4 (33%) HIV-positive patients but in only 1 (10%) control patient. *Blastocystis hominis* was present in the stools of 2 (17%) case-patients and 3 (30%) controls. White blood cells were common in both case-patients (50%) and controls (30%). No pathogenic viruses or bacteria were identified from stool specimens. Small bowel biopsy specimens are currently being examined with electron microscopy for the presence of microsporidia and other potential pathogens. Details of small bowel histopathologic findings will be presented. This study is, to our knowledge, the first to address endoscopic findings in HIV-infected Kenyans. Further studies are needed to better understand the pathophysiology of D/W in Africans infected with HIV.

- TRENDS IN HIV SEROPOSITIVITY IN A PEDIATRIC EMERGENCY WARD PATIENT
502 POPULATION, KINSHASA, ZAIRE. *P. Nguyen-Dinh, N. Shaffer, F. Davachi, K. Hedberg, L. Bongo, F. Behets, A.N. Vernon, Miaka mia Bilenge, and R.W. Ryder. Malaria Branch, Centers for Disease Control, Atlanta, GA, USA; Hopital Mama Yemo, Projet SIDA, and PEV/CCCD, Kinshasa, Zaire.

To evaluate the prevalence and time trends of pediatric HIV infection in Kinshasa, Zaire, a survey was conducted 1-15 November 1988 at Mama Yemo Hospital, the major health facility of the city, and the results were compared with those of a similar survey conducted in 1986. From all children (age <14 years) presenting with medical complaints to the Pediatric Emergency Ward, a medical history, physical examination, and capillary blood sample (for hematocrit, malaria smear, and HIV serology) were collected. Among 1108 children enrolled, the HIV seroprevalence was 4.9%, with the highest rate (12.6%) found in children <6 months old. These results were not significantly different from those of the 1986 survey. Seropositive and seronegative children did not differ in acute symptoms, hematocrit, or malaria parasitemia. The WHO criteria for clinical case definition of AIDS were not accurate predictors for HIV seropositivity in this patient population. As in 1986, HIV seropositivity in children ≥ 1 year was associated with a history of blood transfusion(s); the association was decreased when single transfusions given in 1987 and 1988 only were considered - that is, after the institution of a blood banking and screening program. In conclusion, the HIV seropositivity rate in this patient population has remained relatively stable during the past 2 years, a finding similar to that in adult surveys; further surveys will be needed to confirm the apparent benefits of the recently instituted blood transfusion control measures. Supported by CCCD PASA BST-5927-X-HC-4262.

Y: RETROVIRAL INFECTIONS

503 SEROEPIDEMIOLOGICAL EVIDENCE OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I (HTLV-I) INFECTION AMONG THE HAGAHAI OF PAPUA NEW GUINEA.

R. Yanagihara*, C.L. Jenkins, C. Mora, R.M. Garruto, D.C. Gajdusek. Laboratory of Central Nervous System Studies, National Institutes of Health, Bethesda, MD.

We recently demonstrated high prevalences of HTLV-I infection in several coastal and lowland Melanesian populations having no contact with Japanese, and low seroprevalences in Micronesian populations having such contact. We have now conducted an in-depth seroepidemiological survey for HTLV-I infection among the remote Hagahai, a 260-member, hunter-forager group living at altitudes of 200 to 1800 meters along the northern banks of the Yuat River Gorge in Madang Province in the western Schrader Range of Papua New Guinea. For comparison, two neighboring groups, the Pinai and Haruai, were also studied. Sera were collected between 1985 and 1988. As determined by enzyme-linked immunosorbent assay (ELISA), IgG antibodies against HTLV-I were found in 51% (61/120) of the Hagahai, 46% (31/67) of the Pinai and 36% (31/86) of the Haruai. Among the Hagahai, 45% (10/22) of children (0-9 years), 47% (15/32) of adolescents (10-19 years), and 55% (36/66) of adults (≥ 20 years) were seropositive. Acquisition of infection occurred early, as evidenced by virus-specific antibodies in a one-year old infant, and infection tended to cluster in family groups. By Western immunoblotting, antibodies against HTLV-I gag-encoded proteins p19 and p24 were detected in 97% (59/61) and 28% (17/61), respectively, of ELISA-positive Hagahai sera. Only four individuals, all members of a single household, had antibodies against the major envelope glycoprotein gp46, as well as against both gag proteins p19 and p24. Our data indicate that prototype HTLV-I is present in the Hagahai. In addition, the atypical HTLV-I Western analyses, which are not unlike those we reported for other Melanesian populations, indicate that an antigenic variant of HTLV-I is extant in Papua New Guinea.

504 A RAPID IMMUNOBLOT ASSAY (WESTERN BLOT) TO DETECT SPECIFIC ANTIBODIES FOR HUMAN IMMUNODEFICIENCY VIRUS, *SCHISTOSOMA MANSONI*, AND *TAENIA SOLIUM* (CYSTICERCOSIS).

*Joy A. Brand and Victor C.W. Tsang, Parasitic Diseases Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA 30333, USA

The introduction of the immunoblot into the repertoire of serodiagnostic assays began with the confirmatory testing of human immunodeficiency virus (HIV) antibodies. The difficulty of producing immunoblot strips and the complexity of the time-consuming serum/conjugate incubation steps deterred more widespread use of the immunoblot.

Using the simple Schleicher & Schuell AccutranTM system, can greatly reduce the complexity for strip production and incubation. By simply increasing reagent concentrations, we can reduce incubation times for sera and conjugates (enzyme-labeled second antibodies) to 5 minutes from the usual 1 hour or overnight incubation times. The resulting RAPID BLOT shows no reduction of sensitivity or specificity.

Y: RETROVIRAL INFECTIONS

INACTIVATION OF HIV IN BLOOD SAMPLES STORED AS HIGH SALT
505 LYSATES. *J.W. Zolg, R.S. Lanciotti, and W.A. Meyer III.
Biomedical Research Institute, Rockville, MD and Maryland
Medical Laboratory Inc., Baltimore, MD.

Blood samples to be tested for the presence of P. falciparum DNA using specific DNA probes are routinely collected and stored without refrigeration in form of High Salt Lysates (HSL). Several hundred samples from areas endemic for malaria were analyzed, some of which from patients which were later found to be seropositive for HIV. To safeguard against the risk of accidental infection with HIV while manipulating large numbers of blood samples in preparation for DNA probing, we analyzed the infectivity of HIV after exposure to HSL components.

The toxicity of these components (mainly N-lauroylsarcosine and cesium trifluoroacetate) on the viability of the recipient mononuclear cells used in the long-term propagation of HIV was assessed using the MTT assay. No impairment of cell viability was found in HSL dilutions $\geq 1:4000$. However, a complete inactivation of a high-titer virus stock (tissue culture infective dose: $>10^5$ infective units/ml) was seen after exposure to HSL in dilutions nontoxic for the recipient cells as verified by the absence of any detectable HIV-specific antigens in supernatants of 10 parallel long-term cultures.

This suggests that the virus is in fact completely inactivated by the contact with the lysis reagents, rendering blood specimens stored as HSL noninfective in regard to HIV. (Supported by Contract DPE-0453-A-00-4036-00 from U.S.A.I.D.).

SYMPOSIUM: OPPORTUNISTIC INFECTIONS IN AIDS - NEW INSIGHTS

(No abstracts available)

- 506 INFECTIONS IN AIDS AND THE SEARCH FOR SPECIFIC PNEUMOCYSTIS CARinii ANTIGENS. J.A. Fishman. Massachusetts General Hospital and Harvard Medical School, Boston, MA.
- 507 TOXOPLASMA GONDII: MECHANISMS OF CELLULAR INVASION. K. Joiner. Yale University School of Medicine, New Haven, CT.
- 508 MYCOBACTERIUM TUBERCULOSIS: ASSESSING THE T-CELL RESPONSE. W.H. Boom. University of Cincinnati School of Medicine, Cincinnati, OH.
- 509 CANDIDA: CELL WALL GLYCOPROTEINS IN THE DETERMINATION OF VIRULENCE. J.E. Dormer. Tulane University Medical School, New Orleans, LA.
- 510 DISCUSSION AND CONCLUDING REMARKS. Moderative by J.A. Fishman. Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Z: MALARIA: CHEMOTHERAPY

- 511 MOLECULAR GENETICS OF CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM*. *T.E. Wellems, L.J. Panton, D.J. Krogstad, I.Y. Gluzman, A. Walker-Jonah, V.E. do Rosario, R. Gwadz and S.A. Dolan. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD, and Department of Pathology, Washington University School of Medicine, St. Louis, MO.

The worldwide resurgence of malaria has been accompanied by the spread of chloroquine resistant *Plasmodium falciparum* into nearly all malarious regions. The genetic basis by which the parasite resists the action of chloroquine is unknown. One strategy for identifying the mechanisms involved is a molecular-genetic approach employing crosses of parasite clones and RFLP analysis. To this effect, a cross between a chloroquine sensitive clone (HB3) and a chloroquine resistant clone (Dd2) has been performed. Fifteen independent recombinant clones have been isolated which show an approximately even distribution of chloroquine resistant and chloroquine sensitive forms. The chloroquine phenotype in all resistant progeny is the same as that of the Dd2 parent, while all sensitive forms have that same phenotype as that of the HB3 parent.

To examine whether chloroquine resistance may involve a P-glycoprotein similar to that conferring the *mdr* phenotype in mammalian cells, oligonucleotide probes derived from consensus sequences in the nucleotide-binding folds have been used in conjunction with polymerase chain reaction methods to identify an homologous gene from *P. falciparum*. This gene bears close similarity to the mammalian *mdr* gene and maps to chromosome 5 from the parasite. Linkage data, however, show no correlation between inheritance of the P-glycoprotein and that of the chloroquine phenotype. Likewise, other probes on chromosome 5 show no linkage to chloroquine resistance. Our working hypothesis is that a genetic locus on another chromosome confers resistance. We are performing an RFLP analysis of the parasite chromosomes to search for this linkage group and identify the gene(s) involved in chloroquine resistance.

- 512 TREATMENT OF CHLOROQUINE RESISTANT MALARIA IN *AOTUS* MONKEYS WITH CHLOROQUINE AND A CALCIUM ANTAGONIST. S.K. Martin, *H.L. Williams, V.C. Okoye and D.J. Johnson. Walter Reed Army Institute of Research, Washington, DC 20307-5100.

The mechanism of chloroquine (CQ) resistance in *P. falciparum* is based on a P-glycoprotein related enhanced efflux of CQ from resistant parasites (Krogstad, et al.). Agents that inhibit this efflux process, i.e. calcium antagonists, calmodulin inhibitors, lysosomotropic agents and tricyclic antidepressants, have been shown to reverse CQ resistance in vitro (Martin, et al., 1987, Kyle, et al., 1989, Bitonti, et al., 1988). Therefore, a reversing agent in combination with CQ provides a potential strategy for treating CQ resistant malaria. Six malaria naive *Aotus* monkeys excluding karyotype I were inoculated with 10^6 *Aotus* red cells parasitized with CQ resistant Vietnam Smith *P. falciparum* strain. Parasitemia was monitored daily by giemsa stained thin smear of peripheral blood. When parasitemia reached 1%, an equal number of monkeys were treated with either CQ alone or CQ plus the calcium antagonist, Ro-11/2933. No effect on parasitemia was noted with either treatment schedule. When parasitemia exceeded 15% on either treatment schedule, monkeys were treated for cure with mefloquine. Treatment of CQ resistant malaria using CQ and Ro-11/2933 was not effective in curing monkeys of this disease.

Z: MALARIA: CHEMOTHERAPY

513 EFFECT OF WR-238605 ON THE SPOROGENIC DEVELOPMENT OF PLASMODIUM BERGHEI ANKA IN ANOPHELES STEPHENSI MOSQUITOES.

*R.E. Coleman and R.A. Wirtz. Walter Reed Army Institute of Research, Washington, D.C.

The influence of the antimalarial drug WR-238605 (8-[4-amino-1-methylbutyl] amino]-2,6-dimethoxy-4-methyl-5-(3-tri-fluoromethylphenoxy) quinoline succinate) on the sporogonic development of a Plasmodium berghei ANKA clone was determined. Anopheles stephensi mosquitoes were fed on P. berghei infected mice that had been treated with WR-238605 (25 or 50 mg/kg body weight) 90 min before feeding mosquitoes. Beginning on day 7 post-feed, mosquitoes from each experimental group were dissected every other day (mean number dissected/group/day=41.8) and examined for the presence or absence of oocysts. For each oocyst-positive mosquito, oocyst development was assessed by recording oocyst diameter (mean number of oocysts measured/group/day=46.4). Sporozoite production was then assayed by examining each mosquito for the presence or absence of sporozoites in the hemolymph and salivary glands, while sporozoite infectivity was assessed by allowing mosquitoes from each experimental group to feed on mice. Mosquitoes engorging on mice treated with either 25- or 50 mg/kg WR-238605 produced significantly fewer oocysts/mosquito than did controls, and a smaller percentage of these drug-fed mosquitoes were oocyst-positive. Oocysts in the drug-treated mosquitoes developed more slowly than did those in control mosquitoes (mean oocyst diameter of 16.68 [\pm 1.49] and 13.74 [\pm 0.61] microns on day 15 post-infection for 25- and 50 mg/kg drug-fed mosquitoes, respectively, versus 48.16 [\pm 0.73] microns for control-fed mosquitoes). Sporozoites were not released from oocysts in these drug-fed mosquitoes until day 17 post-infection (vs. day 11 in controls). In no instance did sporozoites successfully invade the salivary glands of drug-treated mosquitoes, nor did these mosquitoes ever transmit P. berghei to mice. Although coincident studies have demonstrated that WR-238605 can affect gametocyte development, the results presented here cannot be attributed solely to gametocytocidal properties of the agent, as no gametocytocidal activity in mice could be detected within 90 minutes (time post drug-inoculation at which mosquitoes were fed) of drug-inoculation. These data therefore suggest that WR-238605 has significant sporontocidal activity and potentially may be used to interrupt malarial transmission.

514 Molecular Basis of Differential Sensitivity to Cycloguanil and Pyrimethamine in Falciparum Malaria.

*David S. Peterson¹, Wilbur K. Milhous², Thomas E. Welles¹. ¹Laboratory of Parasitic Diseases, National Institute Of Allergy and Infectious Diseases, National Institute of Health, Bethesda Md 20892. ²Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington D.C. 20307-5100

The question of cross-resistance between two antifolates, pyrimethamine and cycloguanil is of considerable interest in the chemotherapy of malaria. Clinical reports and field studies have suggested that parasites refractory to cycloguanil (the active metabolite of proguanil) can be treated with pyrimethamine, and vice versa. Studies in our laboratories on the susceptibility of several isolates revealed three pyrimethamine sensitive lines which are resistant to cycloguanil. Nucleotide sequence analysis of the dihydrofolate reductase-thymidylate synthetase gene from these isolates indicates that each carries a point mutation at codon 16. Parasites sensitive to both antifolates encode an Ala at this position while cycloguanil resistant/ pyrimethamine sensitive parasites encode a Val. Alignment of the Plasmodial enzyme sequence with that of both microbial and vertebrate DHFRs shows that Ala 16 is in the β A strand which lies at the back of the active site cavity. Ala 16 is one of 15 residues that have been found to be invariant in all microbial and vertebrate DHFRs studied to date. In the crystal structure of avian DHFR the side chain of this amino acid makes hydrophobic contact with a triazine inhibitor structurally similar to both cycloguanil and pyrimethamine. This suggests that the conservative change from Ala to Val is involved in the differential sensitivity exhibited by these isolates to these two antifolates.

Z: MALARIA: CHEMOTHERAPY

- 515 MALARIA PREVENTION WITH MEFLOROQUINE AMONG PEACE CORPS VOLUNTEERS. *H.O. Lobel, K.W. Bernard, and L.C. Patchen. Malaria Branch and Control Technology Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA, USA; Medical Services, Peace Corps, Washington, D.C., USA.

The spread of chloroquine-resistant Plasmodium falciparum malaria (CRPF) in Africa has increased the risk of acquiring malaria for U.S. travelers because safe and effective drugs were not available for prophylaxis. Among Peace Corps volunteers (PCVs) in Africa, the incidence of malaria in 1988 ranged from 10.8 to 44.4 cases per 100 PCVs, depending on the country and prophylaxis used. The incidence of prophylaxis failures among PCVs suggested that chloroquine no longer provides adequate protection, and the efficacy of chloroquine with proguanil was highly variable. The incidence of imported P. falciparum infections acquired in East Africa among U.S. travelers increased 8-fold since 1978, from 10 to 77 cases. Prophylaxis failures increased from 20% in 1978 to 90% in 1988. Among U.S. travelers to West Africa, the incidence of P. falciparum infections increased 3-fold between 1986 and 1988, from 40 to 124 cases, and prophylaxis failures increased from 18% to 53%.

In May 1989, mefloquine was licensed in the United States but only limited data are available on the incidence of adverse drug reactions (ADRs) associated with prophylactic use of the drug. Mefloquine was made available in May 1989 to PCVs in Africa at high risk of infection with CRPF. To monitor the efficacy and safety of mefloquine prophylaxis, intensified surveillance was initiated among these users. The surveillance includes identification and verification of malaria cases, rapid identification and documentation of severe ADRs, and questionnaire surveys and mefloquine blood-levels every 4 months. The results will be reported.

- 516 P-GLYCOPROTEIN-LIKE MOLECULE IDENTIFIED IN PLASMODIUM FALCIPARUM.

*A. E. Serrano, S. K. Volkman, C. M. Wilson, Z. Etzion, and D. F. Wirth. Harvard School of Public Health, Boston, MA. and Rockefeller University, New York, NY.

Previous work from our laboratory has demonstrated that Plasmodium falciparum contains at least two genes related to the mammalian multiple drug resistant (mdr) genes. One of these genes, Pfmdr1, was shown to be expressed at a higher level by Northern analysis and present in higher copy number by Southern analysis in a strain resistant to multiple drugs as compared to a drug-sensitive parasite strain. A lacZ fusion protein was constructed using a sequenced DNA fragment from the Pfmdr1 gene and pUR292 expression vector. A rabbit antiserum to this fusion protein recognizes a parasite antigen of approximately 150 kilodaltons by Western Blot analysis. Using this antisera the 150 kd antigen appears to be overexpressed in W2mef, a clone drug resistant as compared to its sensitive parent, W2. Initial studies using this antiserum for indirect immunofluorescence localizes this antigen preferentially to the parasite membrane versus the red cell membrane. This provides additional evidence for the similarity of Pfmdr1 to mammalian mdr genes and their expression in resistant cells.

Z: MALARIA: CHEMOTHERAPY

- 517 DNA BINDING BY CHLOROQUINE: POSSIBLE MODE OF ACTION. Frank Kwakye-Berko and Steven Meshnick*. City University of New York Medical School.

The ability of chloroquine to intercalate into DNA was first noted in the 1950's. However, the avidity of chloroquine-DNA binding had not been well characterized. Recently, we measured the binding of chloroquine to DNA by equilibrium dialysis and found that it was highly sensitive to salt concentration, and that it bound avidly enough for 0.03% to 1.0% of potential parasite DNA binding sites to be occupied under physiological conditions (Mol. Biochem. Parasitol. 35:51, 1989). We have now compared the binding of chloroquine to a series of synthetic oligonucleotides and shown that chloroquine does not bind to all sequences of DNA with equal avidity. Chloroquine binds preferentially to the alternating copolymer, poly(dG·dC)·poly(dG·dC), which is of particular interest because of its ability to form left-handed helices, Z-DNA. Chloroquine was found to inhibit the transition of this copolymer from the B to the Z form. Thus, chloroquine may exert its antimalarial effect by binding to specific regions of DNA and causing important alterations in secondary structure.

- 518 IN VITRO REVERSAL OF MEFLOROQUINE AND CHLOROQUINE RESISTANCE IN MULTI-DRUG RESISTANT PLASMODIUM FALCIPARUM ISOLATES FROM THAILAND.

D.E. Kyle*, H.K. Webster, and W.K. Milhous. Walter Reed Army Institute of Research, Washington, D.C. and Armed Forces Institute for the Medical Sciences, Bangkok, Thailand.

Previous studies have demonstrated the existence of two "reversal" phenotypes in drug resistant P. falciparum. Verapamil, chlorpromazine, and desipramine circumvent chloroquine resistance in vitro in all isolates tested thusfar from East and West Africa, Indochina, and South America. Penfluridol reverses mefloquine resistance in vitro in naturally resistant isolates from West Africa. In this study we examined the in vitro reversal of mefloquine and chloroquine resistance in patient isolates from Thailand, an area endemic for multi-drug resistant parasites. Each isolate was found to be resistant to both mefloquine and chloroquine in vitro. Concurrent exposure to mefloquine plus penfluridol (2×10^{-7} M) resulted in a 70-80% reduction in IC_{50} versus mefloquine (from 33.0 to 6.6 ng/ml). The combination of chloroquine plus verapamil (1×10^{-6} M) also resulted in a significant reduction in IC_{50} versus chloroquine (from 62.3 to 8.4). In contrast, penfluridol did not affect the parasite's response to chloroquine and verapamil did not change the response to mefloquine. These results suggest the presence of both "reversal" phenotypes in Southeast Asia.

Z: MALARIA: CHEMOTHERAPY

519 NEW WATER SOLUBLE ARTEMISININ (QINGHAOSU) DERIVATIVES AS ANTIMALARIAL AGENTS

A. J. Lin,* M. Lee, L.-Q. Li, D. L. Klayman, W. K. Milhous and A. L. Ager.¹ Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington D. C. 20307-5100 and Center for Tropical Parasitic Diseases, University of Miami, Miami, FL 33177.

The practical value of artemisinin (qinghaosu), an antimalarial agent isolated from *Artemisia annua*, is impaired by its poor solubility in both oil and water. Sodium artesunate, a water soluble derivative of artemisinin, was developed in China for the treatment of cerebral malaria. The compound, however, has limited stability in aqueous solution. To overcome this problem we have developed three classes of new artemisinin derivatives: 1) ether-linked carboxylates - the carboxylic acid group is used as the solubilizing moiety and is joined to dihydro-artemisinin (DHart) by an ether, rather than an ester, linkage; 2) DHart-amino derivatives - a basic amino function is incorporated into the new molecule to form water soluble acid salts; and 3) DHart-sugar derivatives - a sugar moiety is attached to DHart by an ether linkage to render the molecule more hydrophilic. Among the new carboxylate derivatives, sodium artelinate was found to possess antimalarial activities *in vivo* and *in vitro* comparable to that of artemisinin and sodium artesunate. Sodium artelinate is also considerably more stable in aqueous solution than sodium artesunate. The antimalarial efficacy of these new derivatives have also been evaluated in rodent and simian malaria models.

520 DOES THE INTRINSIC ACTIVITY OF PROGUANIL CONTRIBUTE TO ITS ANTIMALARIAL EFFICACY? C.J. Canfield, K.B. Canfield, B.G. Schuster and W.K. Milhous*. Pharmaceutical Systems Inc., Gaithersburg, MD, and Division of Experimental Therapeutics, WRAIR, Washington, D.C.

Hawking, 1947, 1948, hypothesized that proguanil (PROG) was 'activated' by administration to animals or incubation with minced liver preparations. PROG was the 'pro-drug' & the active component was a putative metabolite. Crounse, 1951, administered PROG to humans and monkeys and found two metabolites: p-chlorophenyl bi-guanide (p-CBG) and a dihydrotriazine, cycloguanil (CYCG). Crowther, 1953, found CYCG ten fold more active than PROG against avian malaria while Schmidt, 1952, found PROG more active than CYCG against simian malaria. Smith, 1961, found that the amount of CYCG produced in rhesus monkeys given PROG was always less than the equivalent amount of CYCG when given alone & concluded that the efficacy of PROG must be due in part to the activity of the parent drug. To validate this conclusion, PROG & its metabolites were assayed in a culture medium containing physiologic levels of folic acid & PABA (Milhous et al., 1985) against three falciparum malaria clones. IC₅₀'s (in ng/ml) were determined as follows:

Parasite Clone	PYR	PROG	p-CBG	CYLOG
Pyrimethamine (PYR) & CYCG Sensitive	00.02	0251.47	0464.47	00.03
CYCG Resistant	00.13	1246.35	2528.27	13.36
Pyrimethamine Resistant	39.49	3204.92	3386.71	00.56

These data not only illustrate intrinsic antimalarial activity, but the IC₅₀ of PROG approaches achievable plasma levels. Since PROG is accumulated 4 fold in erythrocytes (Bygbjerg, 1987), PROG itself may have clinically important activity. Such activity is probably mediated through inhibition of folate metabolism because all compounds were synergistic with sulfonamides and antagonized by folic acid.

Z: MALARIA: CHEMOTHERAPY

- 521 USE OF RIBOSOMAL RNA PROBES TO QUANTITATE MALARIA EXOERYTHROCYTIC DEVELOPMENT AND TO EVALUATE IN VITRO EFFECTS OF CHEMICAL ANTIMALARIALS. J. Li, J. Zhu, T.F. McCutchan, G. Long, A. Appiah, L. Graves, W.K. Milhouse, M.R. Hollingdale. Visiting Scholar, Second Military Medical University, Shanghai, P.R. China; Biomedical Research Institute, Rockville, MD; WRAIR, Washington, DC; NIAID, NIH, Bethesda, MD; NMRI, Bethesda, MD.

A stage specific ribosomal RNA probe has been used to quantitate exoerythrocytic (EE) development of Plasmodium berghei in primary cultures of mouse hepatocytes. As few as 5 EE schizonts could be detected by probe hybridization. During 72 hours incubation, EE-specific rRNA was detected as early as 3 hours after inoculation of sporozoites, and the peak increase was reached at 48 hours. This system was further developed to evaluate the in vitro effects of primaquine and its analogues. Preliminary results showed that after a 48 hour exposure to the antimalarials, the rRNA level of EE parasites decreased in proportion to increased concentration of the drugs. No rRNA could be detected at the concentration achieving complete inhibition of schizont formation (measured microscopically) but causing no cytotoxic effects on host hepatocytes. In contrast to microscopic-based assays, this system can provide an objective and precise in vitro model for rapid screening and evaluation of tissue schizonticidal antimalarial drugs.

- A DIAGNOSTIC TEST FOR CHLOROQUINE RESISTANCE. D.J. Krogstad*,
522 I.Y. Gluzman, P.H. Schlesinger, A.U. Orjih, K. Nkangineme and T.E. Welles. Washington University, St. Louis, MO; University of Port Harcourt, Port Harcourt, NIGERIA; and Laboratory of Parasitic Diseases, NIH, Bethesda, MD.

A test that distinguished rapidly between chloroquine-susceptible and -resistant Plasmodium falciparum would be of great value in the treatment of patients with severe P. falciparum infection.

We have modified the conditions used for the initial studies of chloroquine efflux to approximate those in the infected patient. These differences include: Parasite Stage: the early (ring) stage parasites which circulate in infected patients accumulate 20-fold less chloroquine than the more mature parasites used in previous laboratory studies. Parasite Density: is typically lower in semi-immune patients ($> 4 \times 10^4$ parasites per μ l of blood) than in previous laboratory studies. Other Cells: white cells, platelets and other unparasitized red cells. In addition, estimation of the parasite density is often difficult under field conditions. Verapamil (or other similar agents) may correct for these potentially confounding variables because they enhance chloroquine accumulation only in chloroquine-resistant parasites.

To compensate for these problems, we have used a 10% suspension of the patient's red cells in 50 nM ³H-chloroquine + 10 μ M verapamil. This approach reveals reduced chloroquine accumulation with susceptible P. falciparum and a 10-20% increase in chloroquine accumulation with resistant P. falciparum. Preliminary observations (confirmed by Rieckmann microtiter testing) suggest that 200 μ l of heparinized blood from infected patients may permit one to distinguish between chloroquine-susceptible and -resistant P. falciparum within 3 hours.

SYMPOSIUM: AMERICAN SOCIETY OF TROPICAL VETERINARY MEDICINE

SIGNIFICANCE OF NEW WORLD PRIMATES
IN TROPICAL AND RELATED DISEASES
(No abstracts available)

- 523 INTERNATIONAL TECHNICAL CO-OPERATION ON THE CONSERVATION, REPRODUCTION, AND BIOMEDICAL USE OF TROPICAL PRIMATES. P. Arambulo, PAHO, Washington, DC.
- 524 CYTOGENETICS AND MOLECULAR CYTOGENETICS OF OWL MONKEYS. N. Ma. Harvard University, Cambridge, MA.
- 525 PATHOLOGY OF NON-HUMAN PRIMATES IN RELATION TO PROTOZOAL AND VIRAL DISEASES. R. Broderson. Centers for Disease Control, Atlanta, GA.
- 526 REPRODUCTIVE PHYSIOLOGY OF SQUIRREL MONKEYS. C. Abee. Mobile, Alabama.
- 527 MEDICAL MANAGEMENT OF SPONTANEOUS AND EXPERIMENTAL DISEASES. R.E. Weller. Battelle Laboratories, Richland, WA.
- 528 DISCUSSION: AN OVERVIEW OF THE IMPORTANCE OF NON-HUMAN PRIMATES IN MEDICAL RESEARCH. Introduced and moderated by: W.E. Collins, Centers for Disease Control, Atlanta, GA.

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SESSIONS DESIGNATIONS IN THE AUTHOR INDEX

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ACAV	American Committee on Arthropod Borne Viruses 30th Annual Meeting, Wednesday, December 13, 1:30 PM, South Pacific III-IV.
ACME	5th Annual Meeting of the American Committee on Medical Entomology, Monday, December 11, 1:30 PM, Sea Pearl II-IV.
ADVOCACY	Seminar on the Legislative Process: Advocacy Workshop, Monday, December 11, 5:00 PM, Sea Pearl III-IV.
AIDS	Symposium on Opportunistic Infections in AIDS, Thursday, December 14, 3:00 PM, South Pacific I-II.
ASTMH	American Society of Tropical Medicine and Hygiene Annual Business Meeting, Tuesday, 12 December, 4:30 PM, Coral III-IV.
ASTVM	American Society of Tropical Veterinary Medicine, Thursday, December 14, 1:30 PM, Sea Pearl III-IV.
CTM	Clinical Tropical Medicine Group Meeting, Wednesday, December 13, South Pacific I-II.
CYTO	Symposium on Cytoadherence and Cerebral Malaria, Tuesday, December 12, Coral III.
LATE	Late Breaking Advances in Molecular Biology.
LEPR	Symposium on Hansen's Disease, Thursday, December 14, 1:30 PM, Coral III.

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PLENARY	Plenary Session
PREG	Symposium on Malaria in Pregnancy, Thursday, 14 December, 1:30 PM, Coral IV.
PRES ADDRESS	ASTMH Presidential Address by L.H. Miller, Tuesday, 12 December, 3:30 PM, Coral III-IV.
TRAV	Symposium on International Travel Medicine, Thursday, December 14, 8:00 AM, Coral III-IV.

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